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Developmental changes of seven common cultivars of green peas (Pisum sativum)

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Developmental changes of seven common cultivars of green peas (Pisum sativum)

by

Yu-Hee Kim

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INTRODUCTION

The green pea is the seed of the genus Pisum sativum. The pea is in the Leguminosae family. It is one of the most popular vegetables. In 1969, the pea crop in Canada was valued at seven million dollars (Voisey and Nonnecke, 1973). In the United States the pea crop for 1989 was valued at approximately 119 million dollars (Judge, 1991).

The consumer plays a very important role in influencing directions of the food industry. In a consumer survey, attributes important in selecting vegetables were rated. The survey showed that texture and flavor were the most important sensory attributes, followed by appearance and color (Schultz et al., 1984).

Texture and flavor of green peas can be affected by the following: time of harvest, stage of maturity, processing conditions and the legal chemicals added during processing.

The texture changes in vegetables during maturation and storage are due primarily to the changes in the cell wall components and, to a lesser extent, the storage components deposited in the cytoplasm. In vegetables, tissue differentiation, cell enlargement, and cell-wall growth continue throughout the edible stage. The continued growth and thickening of the cell wall eventually involves lignification and secondary cell wall formation, accounting for most of the chemical aspects of the toughening of vegetables (Van Buren, 1979). Therefore, as peas become overly

mature, they toughen and their quality declines. In peas, this toughening is manifested in the seed coats and as small gritty particles in the cotyledon.

Understanding the magnitude and sequence of biochemical changes occurring in developing green peas should enable scientists to potentially delay the accumulation of textually undesirable carbohydrate polymers; therefore, prolonging the harvest time for commercial processors and resulting in less production of 'extra-standard' peas.

Comparative sensory data on commercial canned peas at different maturities are valuable; however, they are unavailable. Sensory analyses which measure changes in the textural and flavor characteristics of the major genetic pea types might also assist a biotechnology program to develop improved new cultivars.

The Tendrometer is an inexact method for determining harvest maturity in commercially grown peas. Finding a replacement test would be a major technical accomplishment and a benefit to the industry, particularly those persons needing to make decisions regarding economic value or harvesting/processing schedule plans.

The potential for determining one major volatile compound or the change in the total volatiles for a sample of peas as they mature is worth investigating. With the advent of portable gas liquid chromatographs, such analyses are possibile. Further, the possibility of the

volatiles relating to other significant biochemical and physical changes is an interesting prospect. Such indirect indicators of physiological change would help agricultural scientists engaged in crop improvement programs.

To research these possible areas of investigation, two major experiments were designed. In one study, seven cultivars of pea seeds were grown in a defined environment with known nutrients supplied. These cultivars, representing a wide genetic diversity including two round-seeded; one small, wrinkle-seeded freezer type; and four large wrinkle-seeded cultivars, would supply peas for analyses of the biochemical changes, specifically, the pectinmethylesterase enzymatic activity changes, and the volatile changes. As these samples were to be harvested over an extended period, they were also labelled for their maturity from the time of fertilization. The second experiment involved growing four of the seven cultivars on commercial experimental research plots. The two round-seeded, the freezer-type, and one of the commercial, large wrinkle-seeded cultivars were selected. These peas were grown to maturity, harvested, and canned for the sensory analyses. From these efforts, these major "profiles in change" were developed to present in this dissertation.

EXPLANATION OF DISSERTATION FORMAT

The dissertation consists of three manuscripts and two of them were submitted to professional journals. Part I will be published in Journal of Agricultural and Food Chemistry and Part II in Journal of Food Science. Literature cited in the Literature Review of the dissertation are presented in the section, "General References". Literature cited followed the style of the Journal of Agricultural and Food Chemistry. All of the sample growth, harvest, grading, preservation and analyses were carried out by Ms. Kim for all sections of this dissertation. The assistance of Ms. Russey, the graduate student statistics consultant for the College of Family and Consumer Sciences, helped in the design for the sensory analysis study.

LITERATURE REVIEW

Classification of Peas

The garden pea, sometimes called the English, green, or common pea, is a tiny annual cool season plant grown for its edible seed, although some cultivars are also grown for immature edible pods (Valenzuela, 1983). The common commercial cultivars are believed to have evolved from a cross between P. arvense and P. elatius which is often found as a weed in P. sativum crops (Walton, 1988).

Pea cultivars are classified by their growth habit as either vining or bush types. The vining types, except for some edible pea pod cultivars, have limited use. Another category is the pea seed type; both wrinkled and smooth surface seeds are common. Smooth seed types typically have a higher starch-to-sugar content, whereas wrinkled seed types usually have a higher sugar-to-starch content. This relation is a characteristic of fully matured seed. At the edible (immature seed) stage, pea cultivars, whether smooth- or wrinkle-seeded, have a higher sugar content. A belief that small size seed is associated with tenderness and sweetness is incorrect. Large seed can be both sweet and tender. The stage of maturity at harvest rather than size is the determining factor (Hartmann et al., 1988). The cultivar selected is important because the decision about which one to grow not only affects the performance in the field, it also will determine, in part, the quality of the crop that is available to the processor and

hence, the quality of the final product reaching the consumer. In the field, the cultivar determines periodicity or lateness, vine or straw length, resistance to pests and disease, number of pods per node and pod shape (Arthey, 1985).

The correct time for harvesting is very important. The crop is harvested while it is still immature, and, therefore, at a time when its physiological development is very rapid. The optimum maturity for canning peas is the point of development immediately preceding the beginning of the acceleration of the conversion of sugars to starch, when the texture of the peas is still satisfactory without undue toughness of pea skins and firmness of cotyledons (Arthey, 1985).

It can be misleading to assume that the quality of peas can be measured by sieve size alone. As peas mature, they increase in size and starchiness; and, therefore, smallness of the seed is associated with higher quality products. However, some of the best accepted peas from a sensory standpoint come from large sieve sizes and some of the smaller sieve sizes are tasteless when compared to large sieve sizes of the same cultivar (Nonnecke, 1989).

Morphology of Cultivated Peas

The pea seed consists of an embryo (the embryo and two cotyledons) and the seed coat. A small scar, called the hilum, is clearly visible on the seeds. This is the trace of the funicle (ovule stalk) by which the

seed is attached to the ovary wall in the mother plants. The hilum of the pea seed is much shorter than that of vetch.

The hilum of a developing seed is covered with arillus, a part of which is the funicle. The vascular bundle passes through the funicle supplying nutrients from the pod wall. A dot-like opening is located on one side of hilum; this is the micropyle through which the embryonic root emerges in germination.

Two cotyledons enclosed in the seed coat constitute the largest part of the embryo. The stored nutrients required for nourishment in the initial period of growth of the main part of the embryo are concentrated in the cotyledons; the embryo consists of a primioridial root (radicle), stem (collar) and the embryonic growing point (plumule) (Khovstova, 1983).

Chemical Composition

In species P. sativum, the composition of energy yielding components in the fresh green pea are generally as follows: protein 6.3%, carbohydrate 14.4%, fat 0.4% (Nonnecke, 1989).

Protein

Like all grain legumes, because of symbiosis with nodule bacteria, peas accumulate two to three times more protein than cereals. Compared to grain crops, legumes including peas, have the advantage of quantitative as well as qualitative protein content. The biological value of proteins is

firstly determined by the balanced nature of the essential amino acids (they are vitally important for the living organism and can be synthesized only by the plants) and then by the extent of digestibility.

Peas contain all the essential amino acids. Legumes are limiting in the sulfur-containing amino acids, cysteine and methionine, although they are relatively rich in lysine. Overall, the total essential amino acid content of peas compares favorably with that of other legumes (Wright, 1985). The net protein utilization (NPU) value is low and reflects the poor digestibility of legumes in the raw state, but this defect can be easily remedied in dried peas by appropriate heat treatment (Wright, 1985). In addition to protein, the pea seeds also have other nitrogenous compounds, free amino acids, their amides, nucleic acids, peptides, indigenous bases, and inorganic nitrogen (total 2-8%).

Carbohydrate

The carbohydrates in peas are represented mainly by starch (20-50%) and sugars (4-10%). Among the other carbohydrates present are hemicellulose, cellulose, pectin substances and pentoses. The carbohydrate content determines the taste qualities of the seeds, and it is, therefore, important in cultivars grown for food.

The most significant differences in starch content are found between pea varieties with wrinkle seeds and round seeds. The wrinkle seeds

have the lowest starch content. Among the round seeded varieties those with high protein content were characterized by low starch accumulation. On the other hand, the low protein lines had a higher starch synthesis. The round seeded specimens differ significantly from varieties with wrinkled seeds in the type of carbohydrate metabolism occurring during seed maturation. Two main stages can be identified. In the first stage, there is an accumulation of sugars and the low level of starch synthesis. In the second stage, the sugar content drops sharply, while starch content increases. In round-seeded specimens, the first stage is much shorter than in varieties with wrinkle seeds (Makasheva, 1983).

Vitamins

The green peas and immature pods are rich in vitamins. The vitamins of the B-group are well balanced in green peas and immature pods and are accumulated in large quantities. Peas also have the vitamin inositol. The regulatory role of B vitamins and inositol in metabolism are preventing the processes of aging and sclerosis is very great. The ash of pea seeds contains phosphorus, calcium and all the remaining elements (magnesium, sulfur, iron, silicon, chlorine, sodium) (Makasheva, 1983).

Pea-Seed Development and Growth

Growth and expansion of the pod starts after fertilization of the ovules, and the expansion of the pod is complete before significant growth of the seed begins. The pod inflates due to differential growth of inner and outer layers of the cell and form a hollow envelope in which the seed develops and expands (Bryant, 1981).

When the growth of the pod tissues decreases, the growth rate of the seed tissues increase (Bryant, 1981). Pate (1975) has shown that when phases of cell division and cell expansion in the cotyledons are virtually complete, the endospermic fluid has disappeared, and the pools of free sugars and amino acids have been established. The events occur before there is evidence for reserves of starch and protein being laid down in the cotyledons. In Pisum arvense (Flinn and Pate, 1968; Burrows and Carr, 1970) and certain varieties of P. sativum (Carr and Skene, 1961) seed growth is clearly diauxic, the two phases of growth being separated by a short lag of a few days duration, coinciding with the time when the embryo fills the embryo sac. However, Hedley and Smith (1985) classified growth patterns into three phases of rapid growth separated by two short periods of low growth. Most of this initial increase in seed weight was due to the growth of the testa and endosperm. At this stage of seed development, the mass of the embryo is small. The absolute rates of embryo growth were, therefore, low although the relative rate of embryo growth was high. The initial phase of rapid seed growth was followed by a sharp decline or lag phase. This lag in seed growth could

be attributed mainly to a decline in the absolute and relative growth rate of the testa and a decrease in the rate of accumulation of endosperm. This first lag in seed growth was followed by a second rapid growth phase. The relative growth rates of the embryos were very high during this time and in most genotypes, the embryos grew at the expense of the endsoperm, which rapidly declined in volume. Some genotypes, however, maintained a high endosperm volume while the embryos were growing rapidly. This short phase of rapid growth was followed by another lag. A decline in both absolute and relative growth rate was observed in both testa and embryonic tissues. This second lag was followed by a third rapid increase in growth to a maximum followed by a decline as the seed matured and began to dry. Most of the seed growth during this phase was due to embryo development.

Changes in Protein Composition

The pea seed protein, which accumulates during development, may be divided on the basis of solubility charactersitics into two major components, albumins and globulins (Osborne, 1926; Landry and Moureaux, 1970).

The water soluble "albumin" fraction comprises many different proteins. The albumins of Pisum arvense was separated on polyacrylamide gel and resolved into twenty-three bands (Fox, 1964).

The changes in protein content of pea cotyledons have been followed during the period from 9 to 33 days after flowering. Albumins were

synthesized early in cotyledon development whereas globulin synthesis predominated with increasing maturity. At maturity, the pea cotyledon contained approximately 25% protein which was divided into albumins and globulins in the ratio of 1 : 1.4 (Beevers and Poulson, 1971).

Flinn and Pate (1968) also suggested that albumin type proteins accumulate relatively early in the life of embryo and globulin type proteins, by contrast, are laid down only after the cotyledons are almost full size.

Cell Wall Structure

Cell wall structure and components that contribute to texture have been described by Hall (1981) as existing in three-phases. Cell walls are comprised of a crystalline or semi-crystalline fibrillar component, consisting of cellulose; a typically amorphous component, the matrix consisting largely of non-cellulosic polysaccharides but also including significant quantities of protein; and lastly, a packing component. In the primary cell wall the packing component is water, and this component may make up more than half the total fresh weight of the wall. In secondary cell walls the water is replaced by other materials, lignin in the xylem tissue, cellulose in other cases. In some instances various substances such as suberin, cutin and tannins are deposited in the cell wall (Hall, 1981).

The structure of the cell wall of the green pea is a very important contributor to the texture of the peas. Van Buren (1979) writes that cell

walls are comprised of cellulose fibrils embedded in a matrix consisting largely of pectic substances, hemicelluloses, proteins, lignins, lower molecular weight solutes, and water. Northcote (1958) indicated the changing composition and distribution of these components in this classic plant cellwall diagram (Figure 1).

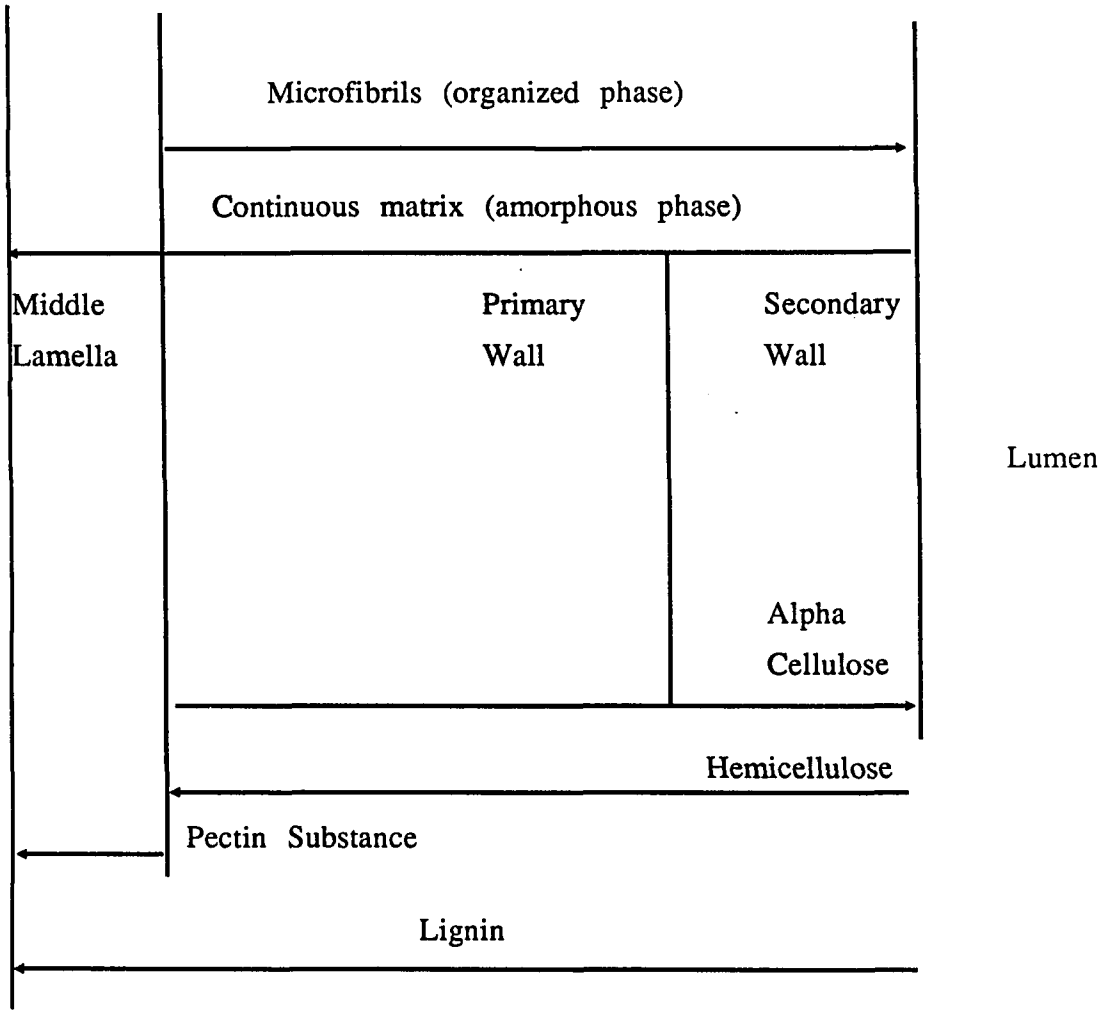

Figure 1. Variations in the concentrations of the major components of plant cell walls (Northcote, 1958)

Cellulose

Cellulose is a high-molecular-weight polymer (DP $\geq$ 10,000) of anhydro-glucopyranose units linked by β(1 --> 4) glycosidic bonds. The chemical resistance and tough native structure of cellulose stems from this molecular ability to establish partially crystalline microfibrils stabilized by mutual hydrogen bonding interactions. Noncrystalline forms of cellulose called amorphous cellulose display little or no fibrous structure as in the case of vegetable pulps (Zapsalis, 1985). Crystalline cellulose fibers provide an important part of the framework of the cell wall of all higher plants (Talmadge et al., 1973). The tenacious hydrogen bonding interactions found in partially crystalline celluloses have a major influence on the behavior and physical properties of cellulose including (1) its characteristic density; (2) its failure to markedly swell in water; and (3) its apparent inert reactivity toward most hydrolytic enzymes and other chemical reagents. Conversely, the absence of comparable hydrogen bonding interactions in amorphous cellulose promotes its swelling in water and decreases the potential tensile strength of its collective microfibrils. This feature of amorphous cellulose structure accounts for its increased elasticity when compared with crystalline forms of cellulose (Zapsalis, 1985).

Hemicellulose

The structural diversity of the hemicelluloses is very great since they offer many variations in molecular branching and are composed of many different sugars and sugar derivatives. Hemicelluloses typically have molecular weights of 600,000, which reflect 150- to 200-unit mixed polymers of hexoses, pentoses, D-galaturonic acid, L-arabinose, 4-0-methyl-D-glucuronic acid, and minor concentrations of L-rhamnose, L-fucose, and various O-methylated neutral sugars. The glucuronic acid residues in particular have an important influence on the solubility of these polysaccharides (Zapsalis, 1985).

Xyloglucan is the major hemicellulose present in the primary cell wall of dicots. Xylosyl residues in the 2-D configuration are linked to carbon-6 of approximately 75% of the glucose residues in the cellulose-like β-glucan backbone. Fucose-galactose, and small amounts of arabinose are also associated with the molecule (Albersheim, 1978; Ring and Selvendran, 1981).

The main or interior chains participate in noncovalent complex formation with the main chains of other polymer molecules. Of particular importance is the ability of xylan (Northcote 1972) and glucans to complex with cellulose, thus attaching the cellulose fibrils to the matrix materials.

The side chain influences the degree of water absorption by the polymer. Arabinose side chains are considered particularly effective in this regard (Northcote, 1972). Rees and Wight (1969) suggested that the side chains interfere with noncovalent interchain bonding. On the other hand, a galactose side chain can become involved in covalent linkages between polymer molecules, further stabilizing the matrix.

Water

Water is a large component of young cell walls. Its contribution to texture can be very profound corresponding to its great complexity of behavior (Frank, 1965). Its influence is easily seen in even the most cursory comparison of the textures of fresh and dried fruits and vegetables. Northcote (1972) has suggested that water plays four major functions in the walls. It is a structural component as part of the matrix gel, it can serve as a wetting agent interrupting direct hydrogen bonding between polymers, it can cooperate in stabilizing conformations of polymers, and it serves as a solvent or milieu for the presence and transport of salts, low molecular weight organic compounds and enzymes (Van Buren, 1979).

The close-packing and orderly arrangement of cellulose molecules in the primary cell wall does not allow the water molecules to penetrate the microfibril regions (Goodwin and Mercer, 1983).

Proteins

Primary cell walls contain 5-10% protein (e.g., Lamport, 1970) which is particularly rich in hydroxyproline (up to 20%), alanine, serine and threonine (Hall, 1981). The hydroxyproline residues are substituted with tetra-arabinosides (Lamport and Miller, 1971) and the galactosyl residues are attached to serine residues (Lamport et al., 1973).

Wall protein is rather resistant to attack by proteolytic enzymes (Muhlethaler, 1967), but treatment of cell walls with proteolytic pronase reduces its tensile strength (Preston, 1974) and liberates pectic and hemicellulose fragments along with peptides from suitably prepared cell walls (Keegstra et al., 1973). Such results indicate that the protein is bound to cell wall polysaccharides. It would appear that the cell wall matrix contains a protein-hemicellulose network (Hall, 1981). It has been suggested that this polymer is linked to the matrix polysaccharides and has a structural role in the primary cell wall (Albersheim, 1978; McNeil et al., 1979; Candy, 1980), or is involved in the synthesis of the cell wall (Candy, 1980).

Pectin

Pectin polysaccharides found in plants are usually bound by calcium in the middle lamella in the growing tissues of many higher plants and to cellulose in the primary cell membrane (Pomeranz and Meloan, 1987).

They can be divided into distinct chemical species and defined as follows:

1. Pectic substances are materials comprising all polygalacturonic acid and containing other carbohydrate materials.

2. Protopectins are water insoluble materials. Restricted hydrolysis of protopectin produces pectinic acids and pectin.

3. Pectinic acids are partly esterified polygalacturonic acids. Those containing a significant number of esterified methyl groups on the polymers will form a gel if acids and sugar concentration are carefully controlled. Low-methoxyl polymers may undergo gel formation with the help of certain metallic ions.

4. Pectins are those water soluble pectinic acids that are capable of gel formation under suitable sugar and acid conditions but may show varying degrees of methyl ester content and degrees of neutralization.

5. Pectic acid is the term used to describe pectic substances composed principally of colloidal polygalacturonic acid that are free from methyl ester groups. Salts of pectic acids are normal or acid pectates (Zapsalis and Beck, 1985).

Classification of Pectin

Pectin can be classified according to the degree of esterification into:

1. High-methoxy pectin (HM): High-methoxy pectin has over 50% DE (the degree of esterification) and gels in a medium with soluble solid content (usually sugar) greater than 55%, at pH range of 2.0-3.5.

2. Low-methoxy pectin (LM): LM pectins have a DE lower than 50%. Gelation is controlled by introducing calcium ions and occurs in a medium with 10-20% soluble solids at pH between 2.5 and 6.5 These pectins make suitable gels when present at 0.5-1.5% (Wang, 1989).

Gel Formation

The bonding between pectin molecules would only be strong if voids could be filled, for example, by small species such as water or ions. The suitable cation cannot only fit the cavity but also screen the electrostatic repulsion between like charges that would otherwise cause the chains to repel (Rees, 1977). This form of association has been named the "egg-box model" because the array of is held between the carbohydrate chains in much the same way that eggs are held between the trays of an egg-box (Grant et al., 1973). An uninterrupted chain length of more than 20 galacturonic acid residues is necessary for binding and the formation of a metal cation-pectate complex (Rees, 1972; Smidsrod and Haug, 1972; Rees, 1977).

A high degree of methylation, the insertion of rhamnosyl residues, and the presence of side chains in the pectin chain interfere with the crosslinking interactions and reduce the capacity of the pectin molecules to associate and form a stable gel structure in the cell wall (Gouled et al., 1965; Rees, 1972; Rees and Welsh, 1977; Bartley and Knee, 1982).

Function and Characteristics of Pectin

Pectin is a component of the cell plate and the cell wall of many growing plant tissues, providing a hydrated and deformable matrix to permit shape change during growth. It represents the cementing substance between the cell walls of mature plant tissues (Rees, 1977).

Portions of the pectic substances of tissues are frequently found to be water soluble under mild extraction conditions and such portions usually have low percentages of neutral sugars and high percentages of methoxylation. Additional pectic material can be brought into solution by the use of chelating agents such as ethylene-diamine-tetraacetic acid (EDTA), sodium hexametaphosphate, or amonimum oxalate-oxalic acid solution. These portions may originally have been insoluble because of Ca^{++} bridges between the adjacent pectic polymers. They usually have lower percentages of methoxylation than do the more easily water solublized pectins in the tissue (Van Buren, 1979).

Other portions can be obtained in the soluble form only after drastic treatments such as the use of elevated temperatures and the presence of

acid or alkali. Possible reasons for these observation include salt linkages, covalent and noncovalent bonding to hemicelluloses, and mechanical intermeshing (Joslyn, 1962).

Heat Processing and Texture

The application of heat has three effects on the tissues of fruits and vegetables. During blanching or the initial heating time, the intercellular air expands and escapes through the exposed surfaces to the atmosphere. With additional heating, the cells are killed and the membranes become permeable to ions which may initiate or enhance chemical and enzymatic reactions. Finally, the cell walls are softened and an increase in deformability and loss in crispness results (Bourne, 1976).

The softening of the tissues during heating is primarily due to the breakdown of pectic substances of the middle lamella. The individual cells may be separated easily without the rupture of the cell walls (Personius and Sharp, 1939; Sterling, 1955; Rockland and Jones, 1974; Bourne, 1976; Loh and Breene, 1982).

Softening of fruits and vegetables during heating appears to occur through two different pectin degrading reactions depending on the pH of the tissue (Van Buren, 1986).

Demethoxylation of the esterified carboxyl groups proceeds under mild alkaline conditions or in mild acid. Splitting of the glycosidic linkages between galacturonic acid residues can take place by a process of

β-elimination, during heating at neutral or alkaline pH. Hydrolysis of glycosidic bonds between neutral sugar residues occurs when pectic substances are heated under mild acid conditions. Splitting of glycosidic bonds of pectic substances embedded in the cell wall matrix increases their solubility (Van Buren, 1979).

Although there is very little direct evidence from this mechanism, indirect support for β-elimination has been described. Conditions that increase the rate of β-elimination also increase the rate of softening during the heating of vegetables (Van Buren, 1986). Higher pH and higher methoxylation content lead to more rapid softening (Lee et al., 1979; Brandt et al., 1984).

The nature and quantity of the cations and anions present in plant tissues can contribute to the texture of heat processed food by their effects on the β-elimination reaction of pectin. The association of cations with the pectin polyanion will decrease its overall negative charge and facilitate the approach of the hydroxyl ions needed to initiate the β-elimination reaction. Citrate, malate, and phytate anions stimulate the β-elimination reaction, while the chloride anion causes very little degradation (Keijbets and Pilnik, 1974a).

The firmness of cooked vegetables can be modified by the addition of salts. Monovalent salts, such as NaCl and KCl, usually soften the product while divalent cations, such as $CaCl_2$, usually firm the product

(Sterling, 1968). The softening of texture by monovalent salts may be due to the displacement of Ca^{++} from the cementing matrix between the plant cells (Dainty et al., 1960). It appears that Ca^{++} has an important role in stabilizing and enhancing the association between pectin polymer chains that is necessary for binding tissue cells together and maintaining tissue firmness (Van Buren, 1986).

Pectin Analysis

Analysis for pectic carbohydrates (polyuronides) in plant materials is difficult because of the varied and complex matrix of non-uronide carbohydrates associated with these samples. Sugars, starches, cellulose and other non-uronide carbohydrates can be present in large quantities in samples such as fruits, juices, vegetables and jellies. Fractional extraction, precipitation, and other separation and purification techniques are commonly used to separate and/or characterize pectic material from these extraneous carbohydrates (Kintner and Van Buren, 1982).

The principal assay utilized to determine uronic acids has been the colorimetric carbazole sulfuric acid reaction by Dische (1947). However, this method is subject to interference from the non-uronide carbohydrates associated with pectin samples. The m-hydroxydiphenyl method is less sensitive to extraneous carbohydrate interference (Blumenkrantz and Asboe-Hansen, 1973). However, before using this method for pectin analysis, the amounts of non-uronide carbohydrates should be calculated in

the sample. If final sample dilutions contain more than 200 μg/ml of non-uronide material, then a purification step will give a more accurate uronide value when using this assay (Kintner and Van Buren, 1982).

Pectinmethylesterase

Ripening fruit softens because pectin and other cell wall carbohydrates are broken down enzymatically. Pectinmethylesterase (PME, pectin pectyl-hydrolase, EC 3.1.1.11) removes methoxyl groups from methylated pectic substances (pectin) and, therefore, belongs to the sub-division of enzymes which hydrolyze carboxylic acid esters. Most pectin esterases initiate attack on pectin at a position adjacent to a pre-existing free carboxyl group (Whitaker, 1972).

In vegetables, the maturity level of plant was described as the major factor influencing PME activity in southern peas (Vigna sinensis). The more immature peas, the higher the activity (Collins, 1970). Pectinesteresases have been found to be present in the species of higher plants (Lineweaver and Jansen, 1951). A number of plant pathogenic, fungi and bacteria are known to produce pectinesterase (Rexova and Markovic, 1976; Wang, 1989). All pectinesterases are highly specific for methyl esters of polygalacturonate (Rexova and Markovi, 1976).

Methyl esters of other uronides or polymers of less than ten galacturonic acids are not de-esterified. De-esterification starts from the reducing end or at some secondary locus, next to free carboxyl groups,

and proceeds along the chain, creating blocks, of free carboxyl groups (Miller and MacMillan, 1971). In the action of pectinesterase, end-product inhibition takes place. The product, polygalacturonic acid, acts as a competitive inhibitor in tomato pectinesterase (Lee and MacMillan, 1968).

The activity of pectinesterase is affected by pH, temperature, presence of salts and various inhibitors (Rexova and Markovic, 1976). Most plant pectinesterases have pH optima between 7 and 9 (Rexova and Markovic, 1976; Wang, 1989). The presence of salts of monovalent and divalent cations increases the activity of pectinesterases from higher plants, by several fold, which is minimal in the absence of salts (Van Buren et al., 1962; Lee and MacMillan, 1968).

Chemical Assay of PME

Several methods have been described for determining the products of pectin hydrolysis. The methanol which is produced on hydrolysis can be determined chromatographically (McFeeters and Armstrong, 1984) or colorimetrically (Wood and Siddiqut, 1971). Although the chromatographic method for methanol determination is very sensitive, it is not convenient for routine enzyme determination. The colorimetric methods require large volumes of reactants and are time consuming (Hagerman and Austin, 1986). As an alternative method, the continuous spectrophotometric assay is based on the color change of a pH indicator during the PME-catalyzed reaction. As the ester bonds are hydrolyzed, acid groups are produced

which dissociate and the pH is lowered, causing the indicator dye to change color (Hagerman and Austin, 1986).

Flavor Compounds

Flavor has been defined as "the sum of those characteristics of a material taken into the mouth, perceived principally by the senses of taste and smell, and also be the general pain, tactile and temperature receptors in the mouth, as received and interpreted by the brain" (Teranishi et al., 1971). True or basic taste is defined using the terms sweet, bitter, sour, and/or salty, where as taste sensations are described using terms such as cooling, burning, pungent, or biting (Pruthi, 1980).

Natural and fabricated foods are complex and usually non-uniform systems. The perceived flavor of these foods is the result, in practically every case, of the combined sensation caused by numerous minor and major classes of flavor compounds.

The total amount of flavor compounds in food is very small. The quantity of flavor material in a natural raw food ranges from 100 parts per million (ppm) to only a few ppm. For example, bananas have 12 to 18 ppm of flavor volatiles, raspberries 2 to 5 ppm, strawberries 2 to 88 ppm, tomatoes 3 to 5 ppm, beef 30 to 40 ppm and cocoa around 100 ppm. In a prepared food, the concentration of flavor compounds may be only a few parts per billions (ppb) (Emberger, 1985).

Sensory Characteristics of Flavor Compounds

According to Heath and Reinecius (1986), each of these types of flavor compounds has varying sensory character. Lower molecular weight aldehydes have an unpleasant odor. Higher weight ones have a pleasing fruity character. Dilution also plays a role in determining the flavor sensation imparted by a chemical. Aldehydes with 8 to 10 carbons, though bitter at high concentrations, become floral upon dilution. Alcohols are among the most important of flavoring material and are extensively found in nature. Lower molecular weight alcohols have a sweet odor, while ones with higher molecular weights are unpleasant. Higher molecular weight ketones, starting with C-7, are widely used in imitation flavorings. As the carbon number increases, the fruity odor changes to a floral note. Esters vary in their character. Each one must be considered individually; however, overall they have a fruity note.

Headspace Method

Quantitative headspace analysis of volatile organic compounds in equilibrium with good ingredients can be used as a valuable tool (Saleeb and Pickup, 1978).

Headspace methods can be divided into two general classes, direct injection and headspace concentration. The direct injection method is simple and rapid, and theoretically, samples only what the nose receives. Sample handling is minimized, thereby reducing the probability of artifact

formation. Unfortunately, only those substances whose vapor pressures are sufficiently high that they are present in headspace in amounts large enough to activate the detector will produce peaks (Jennings and Shibamoto, 1982; Heath and Reineccius, 1986).

Trace analyses of food volatiles may be accomplished via headspace concentration technique which includes cryogenic techniques.

Cryogenic Techniques in Gas Chromatography

There are three types of cryogenic techniques as they are known today: cryogenic gas chromatography (CGC), cryogenic focusing (CF), and cryogenic trapping (CT).

Cryogenic gas chromatography Cryogenic gas chromatography (CGC) at temperature below ambient is best known for the separation of gaseous mixtures (Brettel and Grob, 1985). Cryogenic techniques often significantly improve separation of volatiles as noticed by Thompson (1977) for subambient separation of Ar and O_2, and hydrocarbons as reported by Adlard et al. (1979), and Osgard and Malthe-Soerenssen (1983).

Cryogenic focusing Cryogenic focusing, commonly known as "cryofocusing", uses the cold temperatures of cryogenic coolants to focus the sample into a plug at or near the head of the column for the purpose of improving the peak shape (Brettel and Grob, 1985). The principle of cryogenic focusing, described by Takeeka and Jennings (1984), is that in colder temperatures, the front of the band moves more slowly

than the rear and the solute band is sharpened or focused. Cryofocusing has been used in a variety of analytical methods, monitoring trace level organics in ambient air (Cox and Earp, 1982), measuring hazardous organic emissions (Krost et al., 1982), and quantitating volatile urinary metabolites (Cert and Bahimas, 1984).

Cryogenic trapping Cryogenic trapping can be described as the trapping of volatile components in solution or on adsorbents primarily for enrichment purposes by passing the headspace or purge gas through a series of cold traps or absorbent materials (Brettel and Grob, 1985; Heath and Reineccius, 1986).

A major problem with cryogenic trapping is that water is the most abundant volatile in most food and, therefore, the trap condensate is primarily water. The trapping of volatile flavor on an absorbent which has minimal affinity for water eliminates the need for solvent extraction and associated problems (Heath and Reineccius, 1986). The use of synthetic porous polymers for trapping of volatiles from headspace has become the most commonly used absorbent (Charlambous, 1978).

Part I. PECTINMETHYLESTERASE ACTIVITY CHANGES DURING THE DEVELOPMENT OF THE GREEN PEA (Pisum sativum) TO HARVEST MATURITY

Pectinmethylesterase Activity Changes During the Development of the Green Pea (*Pisum sativum*) to Harvest Maturity*

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ABSTRACT

Understanding the magnitude and sequence of biochemical changes occurring in developing green peas should enable scientists to potentially delay the accumulation of textually undesirable carbohydrate polymers; therefore, prolonging the harvest time for commercial processors and resulting in less production of 'extra-standard' peas. Toward understanding these processes, the content of pectin methylesterase (E.C. 3.1.1.11) (PME) in seven commercial cultivars of greenhouse-grown, green peas from 12 days after fertilization until harvest maturity (21-22 days) was monitored. PME specific activity decreased in all cultivars at an apparent first order rate constant of 17% per day. During the same period, the protein content was accumulating. Two of the wrinkle seeded cultivars showed changes in chelate soluble pectin of less than 20% (W-2, W-3). The greatest decreases of chelate soluble pectin were exhibited by R-2 and W-1.

INTRODUCTION

Pectinmethylesterases (PME) (Pectin pectyl-hydrolase, EC 3.1.1.11) have been found in all species of higher plants -- in their fruits, leaves, stems, and roots. A substantial proportion of PME is bound to the cell wall (Lineweaver et al., 1951). Pectinmethylesterase can hydrolyze the galacturonic acid methyl esters by de-esterifying the pectin substances, which are composed of α-1, 4-linked galacturonic acid and galacturonic acid methyl ester (Rexova-Benkora and Markovic, 1976). In a study of green peas, one important sensory characteristic is texture and PME has been shown to influence it (Schultz et al., 1984).

The activity of PME is affected by pH, temperature, presence of salts, various inhibitors (Rexova and Markovic, 1976), and the maturity of the plant tissue (Lineweaver et al., 1951). Most plant PME's have a pH optima between 7 and 9 (Rexova and Markovic, 1976; Wang, 1989). NaCl increases the in vitro maximum activity of PME by several fold in extracts from higher plants (Van Buren et al., 1962; Lee and Macmillan, 1968). Maturity of the plant tissue also may influence relative PME activity (Lineweaver et al., 1951). An increase in enzymatic activity with maturity has been demonstrated in oranges by Rouse et al. (1962) and in cherries by Al-Delaimy et al. (1966).

The texture changes in vegetables during of maturation and storage are due primarily to the changes in the cell wall components and to a

lesser extent the storage compounds deposited in the cytoplasm. In vegetables, tissue differentiation, cell enlargement, and cell-wall growth continue throughout the edible stage. The continued growth and thickening of the cell wall eventually involves lignification and secondary cell wall formation, accounting for most of the chemical aspects of the toughening of vegetables (Van Buren, 1979). Therefore, as peas become overly mature, the seed coats toughen and their quality as a vegetable declines. High levels of graininess, seed-coat toughness, dry mouthfeel, and mushy consistency were observed in cooked green peas by Grillier (1989).

The texture of green peas can be affected by the following features: time of harvest, stage of maturity, amount of PME activity, processing conditions, and chemicals, legally added during processing.

The question raised in this study is whether there is an opportunity for the PME to influence the texture of thermally processed commercial peas. Specifically, the activity change during development toward commercially harvestable maturity may present the opportunity to use PME activity, in conjunction with the optimum blanching temperatures, to enhance the final texture of processed pea. No specific studies relative to the changes in PME activity during development of green peas were found to document the activity changes.

This study is designed to profile changes in commercial green pea PME activity and other related constituents from 12 days after fertilization (DAF) until commercial maturity (21-22 days). The objectives were: 1) to optimize the assay for pectinmethylesterase (PME) in terms of ionic strength of the extraction medium, temperature, and critical timing consideration for the determination; 2) to observe the changes in PME; and 3) to monitor other chemical changes playing roles in final product texture.

MATERIALS AND METHODS

Sample Growing

Seven cultivars of green peas were raised in the Ames greenhouse by the Department of Horticulture. The greenhouse was maintained to simulate cool-season growing conditions by using supplemented lighting and temperature controls. The following seven cultivars were used in this study: a wrinkle-seeded small sieve, dark-skinned freezer pea (F); two round-seeded, high starch pea types (R-1, R-2); and four large sieve, wrinkle-seeded pea types of different growing seasons (W-1, -2, -3, -4). Plants were monitored daily until flowers showed bending of the first petal back toward the stem, which was taken as the time of fertilization. The pea plants were tagged with the Julian date and allowed to grow until nearly senescent. All varieties were harvested by hand from 12 to 22 days after fertilization (DAF). The peas were shelled by hand, size graded, packaged, and frozen with liquid nitrogen. The frozen peas were stored in the freezer at -75 ^{o}C.

Given the fact that all possible sieve sizes were not available and in an interest of gaining the profile of the activity changes without consuming all of the material we had grown the PME activity, protein, and moisture analyses were performed for four cultivars on an even-numbered day basis and for three cultivars on an odd-numbered day basis. Limitations of inventory was a major constraint in this decision.

Procedure for PME Activity

Reagents

A 0.5% (w/v) solution of citrus pectin (Sigma Chemical Co.) was prepared in distilled water by heating (40 °C) the mixture during continuous stirring. A 0.01% (w/v) solution of bromothymol blue was prepared in 0.003 M, pH 7.5 potassium phosphate buffer. All solutions (pectin, indicator dye, water) were adjusted to pH 7.5 with dilute HCl and NaOH before each trial began.

Sample extraction

Pectin methyl esterase activity assay parameters were established through an initial study. Optimum levels of NaCl concentration and extract holding time before assaying were established. The NaCl concentration for the extraction media was varied from 0-1.5 M, and the holding time after extraction was varied from 0-3 hours. The activities were measured. The time and concentration chosen provided optimum activity and efficient logistics for conducting the assay. These became the conditions set out below.

The green peas (4.5 g) were homogenized with cold 1 M NaCl (15 mL) on a tissuemizer homogenizer and stirred on a magnetic stirrer for 1 hour at 2 °C. The homogenates were centrifuged for 20 min at

27,500 x g to remove large particles and pigments. The pellet was discarded and the supernatant recentrifuged for an additional 20 min at 27,500 x g. The supernatant was collected and adjusted to pH 7.5 with dilute HCl and NaOH (Summers, 1989).

Determination of pectinmethylestease activity

The spectrophotometric assay was calibrated with galacturonic acid (Hagerman and Austin, 1986). The reaction was monitored at 620 nm in a Gilford 240 recording spectrophotometer (Oberlin, Ohio). The temperature was maintained at 25 ^{o}C, with a circulating water bath. The assay was carried out in the cuvette. Pectin (2.0 mL) was mixed with 0.15 mL of bromothymol blue and 0.75 mL of water. The reaction was started with the introduction of 0.1 mL of plant extract containing the enzyme. The rate of decrease in absorbance at 620 nm was recorded (Hagerman and Austin, 1986). A unit of PME is an amount of enzyme that releases 1μ mol of acid/min per mL of extract.

Procedure for Moisture

The moisture content of the green pea was determined by drying samples in duplicate for 65 hours at 60 ^{o}C in a hot-air convection oven (AOAC, 1955).

Procedure for Pectin

The amount of pectin was determined by the m-hydroxydiphenyl method of Kintner and Van Buren (1982). Samples were blended with deionized water with a Tekmar tissuemizer, followed by centrifugation and filtration. Water soluble pectin extract was obtained and the oxalate soluble pectin extracted with a solution of ammonium oxalate and oxalic acid (Dekker and Richards, 1972). The absorbance of the samples, following chromagen formation, was measured at 520 nm using a Beckman DU spectrophotometer equipped with an Update.

Procedures for Protein

Soluble protein contents in the NaCl extracts for PME activity were measured according to the Biuret method (Gornall et al., 1949). 0.1 mL of the extract was incubated with Biuret reagent for 30 min at room temperature and read at 450 nm on a Beckman DU equipped with an Update. These data were essential for the expression of the specific activity in these extracts.

Data Analyses

The statistics analytical software package Statistix (version 3.1) was used in all statistical analyses (Analytical Software, 1989). PME activity measurements, dry matter analyses, pectin measurement, and biuret protein contents were performed in duplicate for all selected samples. The peas samples were collected from one planting of each of the cultivars.

RESULTS AND DISCUSSION

The effects of NaCl concentration and of length of holding of plant extract slurry on PME activity are presented in Tables 1 and 2. Maximum activity for PME in one of the cultivars used in the initial study was obtained at 1 M NaCl and did not increase at higher salt levels. A lower level of salt reduced the PME activity. Table 2 shows the changes in PME activity when the slurries of peas were allowed to stand for up to 3 hours. There was progressive increase in activity for 1 hour, but no significant changes occurred from 1-3 hours.

The rates of changes in PME-specific activity are shown in Table 3. PME activity decreased over all varieties and all sieve sizes. The mean rate of activity loss followed an apparent first-order rate model. Mean 'k' (apparent first order rate constant) was 0.168 Day^{-1}. The freezer type variety (F) had the highest k ($k = 0.25$); whereas, one of the round seeded varieties (R-2) had the lowest rate ($k = 0.11$).

As the peas matured, salt soluble proteins accumulated and PME activity decreased. The more immature the peas, the higher the activity. A three fold change in activity was seen over the maturity levels for an individual cultivar and across all cultivars. Although Collins (1970) has shown that more mature southern peas (Vigna sinensis) exhibit less PME activity, that study did not document the

relation of maturity and quality parameters to the changes in PME activity as extensively as the current study.

All varieties exhibited the accumulation of salt-soluble protein. The accumulation of protein showed a strong correlation with maturity (Table 4). The amount of protein increased 2-4 times as maturity level increased from 12-20 DAF (Table 4). Each variety had different patterns of the protein accumulation, however. There was no consistent trend within any of these sample types. As the pea seeds matured (greater age at a particular size), they accumulated salt soluble proteins. These proteins are indicative of globulins and not albumins, which are largely enzymes (Pate, 1985). Viewed in this context along with the knowledge that vegetable peas are the immature form of the tissues which will become the seed, loss of PME activity would be expected within the normal development of the plant.

Changes in water soluble and chelate soluble pectin content are shown in Table 5. Each cultivar contained different amounts of pectin. Comparing changes in chelate soluble pectin over the time of the study, two of the wrinkle-seeded cultivars showed changes of less than 20% (W-2, W-3). The greatest decreases of chelate soluble pectin were exhibited by R-2 and W-1. Earlier work on the development of peas (Pate, 1985) gave no indication of this loss of pectin content. Given the stage of development exhibited by the peas, however, with

little or no cell division occurring, the dilution or loss of pectin as other cell-wall materials (Pate, 1985; Grillier, 1989) are accumulated would be reasonable. There was no distinct difference in pectin content from cultivar to cultivar or between wrinkled or smooth seed types.

The moisture level was inversely related to the maturity level of these samples. As expected, the sieve size increased as did the dry matter ($r = 0.80$), and the water loss ($r = 0.90$) increased also. Each seed type showed either a biphasic or a linear relation between dry matter accumulation and the maturity index (DAF).

Insufficient material was produced from many of the samples on days for which PME and the other compositional information were taken to provide a sample for texture analyses in the Ottawa Texture Cell on an Instron instrument. However, the seven cultivars have known widely different textural characteristics. The freezer-type (cultivar F) is recognized as a firm, small-seeded type pea. The round- seeded types are forage type peas known for their starch accumulation, and they have a pasty texture. The wrinkle-seeded peas are all high-sugar large-seeded peas of intermediate firmness. Four of these cultivars (R-1, R-2, F, W-1) were grown commercially and processed in a commercial pilot plant as samples for another study.

Sensory analyses of these samples ranked them in order of decreasing firmness as F, R-1, R-2, W-1 (Kim et al. in preparation).

The high chelate soluble pectin content of cultivar F (sieve size 2) and the R-1 samples could certainly account for their texture rankings in the sensory study. Insufficient material was available for calcium analyses of these samples; however, previous work in our lab (Grillier, 1989) with two cultivars of peas showed decreased calcium content in both cultivars studied as the peas matured. The peas showed increased losses in PME activity, and decreased chelate pectin content. These facts along with the known accumulation of starch and non-pectin carbohydrates and the decrease in calcium would leave little opportunity for expecting a PME/chelate pectin system that could be used post-harvest to provide enhanced firmness in commercially mature peas.

Table 1. Activity of PME at different concentrations of NaCl, measured at 620 nm, pH 7.5, 25 °C, after 1 hour of holding

M	PME Activity (1 μmol/min/mL extract)
0	0.019
0.25	0.038
0.5	0.077
1.0	0.231
1.5	0.231

Table 2. PME-activity at different slurry holding times, measured at 620 nm, pH 7.5, 25 °C, 1.0 M NaCl

Time (hr.)	PME Activity (1 μmol/min/mL extract)
0	0.385
1	0.550
2	0.539
3	0.566

Table 3. Rates of changes in PME-specific activity

Cultivar[a]	Sieve Size	Rate of Loss in Specific Act[b]	Goodness of Fit First Order Model r	Probability
R-1	3	0.20	0.94	0.0006
R-2	3	0.11	0.94	0.0001
W-1	3	0.13	0.81	0.0150
	4	0.14	0.65	0.1618[c]
W-2	5	0.15	0.91	0.0019
	6	0.17	0.64	0.1705[c]
W-3	3	0.18	0.78	0.0688[c]
	4	0.13	0.98	0.0201
W-4	3	0.23	0.94	0.0006
	5	0.15	0.69	0.1314[c]
F	2	0.25	0.96	0.0018
	3	0.18	0.75	0.0858[c]

[a]R-1, R-2 = round-seeded cultivars;
F = small freezer-type cultivar;
W-1, W-2, W-3, W-4 = wrinkle-seeded cultivars.
[b]Apparent First Order Rate Constant, 'k' Day^{-1}.
[c]Not significant at $P \leq 0.05$ for H_o: $r = 0$.

Table 4. Changes of salt soluble protein content in the 1.0 M NaCl extracts of peas for PME activity (mG/mL)

Cultivar[a]	Sieve Size	DAF[b]	Protein (mG/mL)[c]
R-1	3	13	4.44 ± 0.26
		15	7.80 ± 0.35
		17	10.17 ± 0.26
		19	11.09 ± 0.52
R-2	3	12	6.52 ± 2.16
		14	7.86 ± 0.61
		16	9.07 ± 0.07
		18	11.82 ± 0.69
		20	12.62 ± 0.09
F	2	12	4.14 ± 0.69
		14	5.42 ± 1.29
		16	7.56 ± 0.35
	3	16	8.94 ± 0.11
		18	10.97 ± 0.17
		20	12.61 ± 0.96
W-1	3	16	6.64 ± 0.43
		18	8.53 ± 0.52
		20	11.27 ± 0.25
		22	11.88 ± 0.26
	4	18	9.08 ± 1.98
		20	12.79 ± 0.35
		22	14.99 ± 0.52

[a]R-1, R-2 = round-seeded cultivars;
F = small freezer-type cultivar;
W-1, W-2, W-3, W-4 = wrinkle-seeded cultivars.

[b]DAF = days after fertilization.

[c]Within a sieve size bound by a bracket, significance of the change in each cultivar is indicated for $P \leq 0.05$, H_o: $r = 0$; NS for $p > 0.05$.

Table 4. Continued.

Cultivar[a]	Sieve Size	DAF[b]	Protein (mG/mL)[c]	
W-2	5	12	5.24 ± 0.18	
		14	7.56 ± 0.52	
		16	7.43 ± 1.70	
		18	9.14 ± 0.43	
	6	16	9.57 ± 0.43	
		18	10.84 ± 0.35	
		20	13.89 ± 3.79	NS
W-3	3	13	7.00 ± 1.29	
		15	8.84 ± 0.43	
		17	9.51 ± 0.69	
	4	15	9.81 ± 0.08	
		20	11.64 ± 0.95	NS
W-4	3	13	3.47 ± 0.60	
		15	6.15 ± 0.43	
		17	8.29 ± 0.35	
		20	12.28 ± 2.10	
	5	13	6.33 ± 0.17	
		15	7.86 ± 2.33	NS
		17	9.08 ± 1.46	

Table 5. Changes in water soluble pectin and chelate soluble pectin

Cultivar	Sieve Size	DAF[a]	Water Sol. Pectin (mg/g dry peas)	Chelate Sol. Pectin (mg/g dry peas)
R-1	3	13	3.99 ± 0.21	17.61 ± 1.67
	3	19	1.74 ± 0.04	9.64 ± 1.20
R-2	3	12	2.71 ± 0.06	10.03 ± 0.39
	3	18	1.08 ± 0.06	5.01 ± 0.02
F	2	12	4.04 ± 0.15	17.02 ± 2.09[b]
	2	16	1.72 ± 0.07	11.31 ± 1.80[b]
	3	16	1.64 ± 0.03[b]	9.36 ± 0.42
	3	20	1.71 ± 0.02[b]	5.80 ± 0.06
W-1	3	14	5.09 ± 0.04	16.45 ± 0.96
	3	20	1.60 ± 0.06	7.95 ± 0.20
W-2	5	12	3.04 ± 0.25	11.10 ± 0.57
	5	14	3.14 ± 0.91	10.85 ± 1.38
	5	16	2.47 ± 0.17	9.88 ± 1.09
	5	18	2.16 ± 0.03	8.88 ± 0.59
W-3	3	13	2.92 ± 0.04[b]	15.28 ± 0.18[b]
	3	17	2.81 ± 0.14[b]	12.94 ± 2.00[b]
W-4	3	13	5.54 ± 0.23	17.37 ± 0.92
	3	20	1.32 ± 0.02	10.96 ± 0.55

[a]DAF = days after fertilization.

[b]Pectin changes within a sieve size are not significant at $P \leq 0.05$, H_o: $r = 0$.

CONCLUSION

PME-specific activity decreased over all pea cultivars and all sieve sizes. The rate of activity loss followed an apparent first order rate model. Mean 'k' was 0.168 $(day)^{-1}$. As the peas matured, salt soluble protein accumulated and PME activity decreased. This study represents the first detailed report of the changes in this important pectin modifying enzyme during green pea development to harvestable maturity.

Given the differences in seed types, it was not surprising that each cultivar contained different amounts of pectin. The greatest decreases of chelate soluble pectin were exhibited by R-2 and W-1.

Salt-soluble protein accumulated over all cultivars. The moisture level decreased as maturity increased, which inversely reflected the increase in protein.

REFERENCES

Al-Delaimy, K. A.; Borgstrom, G.; Bedford, C. L. Pectic substances and pectic enzymes of fresh and processed montomorency cherries. Mich. Agric. Exp. Stn. Q. Bull. 1966, 49, 164.

Analytical Software, Statistix, 1958 Eldridge Avenue, Roseville, MN. 1989.

AOAC. In Official methods of analysis, 8th ed.; Association of official agricultural chemist, Washington, D.C., 1955.

Collins, J. L. Pectin methylesterase activity in southern peas. J. Food Sci. 1970, 35, 1-4.

Dekker, R. F. H.; Richard, G. N. Determination of pectic substances in plant material. J. Sci. Food Agric. 1972, 23, 475-483.

Gornall, A. G.; Bardawill, C. J.; David, M. M. Determination of serum proteins by means of the biuret reaction. J. Biol. Chem. 1949, 177, 751.

Grillier, D. E. Physicochemical and sensory analyses of optimal and extra-standard quality peas. M.S. Thesis, Parks Library, Iowa State University, Ames, IA. 1989. (Unpublished).

Hagerman, A. E.; Austin, P. J. Continuous spectrophotometric assay for plant pectin methylesterase. J. Agric. Food Chem. 1986, 34, 440-444.

Kintner, P.; Van Buren, J. Carbohydrate interferences and its correction in pectin analysis using m-hydroxydiphenyl method. J. Food Sci. 1982, 47, 745-759.

Lee, M.; Macmillan, J. D. Mode of action of pectic enzymes. I. Purification and certain properties of tomato pectinesterase. Biochemistry 1968, 7, 4005-4010.

Lineweaver, H.; Jansen, E. F. Petic enzymes. In Advances in enzymology and related subjects of biochemistry; Nord, E. F., Ed.; Interscience: NY, 1951; p. 267.

Pate, J. S. In The pea crop; Hebblethwaite, P. D., Heath, M. C., Dawkins, T. C. K., Eds.; Butterworths: England, 1985; p. 334.

Rexova-Benkova, L.; Markovic, O. In Advances in carbohydrates chemistry and biochemistry; Tipson, R. S., Horton, D., Eds; Academic Press: NY, 1976; p. 323.

Rouse, A. H.; Atkins, C. D.; Moor, E. L. Seasonal changes occurring in the pectinesterase activity and pectic constituents of the component parts of citrus fruits. I. Valencia oranges. J. Food Sci. 1962, 27, 419.

Schultz, H.; Martens, M.; Rodbottem, M.; Wilshewr, B. Consumer perception of vegetable quality. Acta Hortic. 1984, 163, 31-38.

Summers, W. L. Pectinesterase and D-galacturonase activities in eight snap bean cultivars. Hort Science 1989, 3, 484-486.

Van Buren, J. P. The chemistry of texture in fruits and vegetables. J. Texture Studies 1979, 10, 1-23.

Van Buren, J. P.; Moyer, J. C.; Robinson, W. B. Pectin methylesterase in snap beans. J. Food Sci. 1962, 27, 291.

Wang, D., Ed. Mechanism and theory in food chemistry; Van Nostrand Reinhold: NY, 1989; p. 105.

Part II. COMPARATIVE SENSORY ANALYSES OF TEXTURE AND FLAVOR CHARACTERISTICS OF FOUR COMMON CULTIVARS OF GREEN PEAS (Pisum sativum)

ABSTRACT

Processed peas from four cultivars, one large wrinkle-seeded, two round-seeded, and one small-seeded freezer type, were evaluated by a thirteen-member trained panel. Using an unstructured 15 cm line the judges measured texture attributes and flavor characteristics at immature, optimally mature, and overly mature stages of development. A partially balanced incomplete block sensory-study design was employed and an analysis of variance showed variety and maturity differences for firmness, pastiness, tough seed coat, and sweetness. The freezer-type pea was the firmest and least pasty, having the toughest seed coats. The wrinkle-seeded type was the sweetest.

INTRODUCTION

Pea cultivars can be classified by seed type; both wrinkled and round surface seeds are common. Wrinkle-seeded cultivars typically have a higher sugar to starch content than round-seeded cultivars. Canned peas are processed from mainly two varieties which are the wrinkle-seeded and the smooth-seeded (Aleksic and Luh, 1975).

It has been known that the optimum maturity for a canning pea is the beginning of the acceleration of the conversion of sugars to starch without toughening of the seedcoat (Arthey, 1985). At the edible (immature seed) stage, pea cultivars, whether round- or wrinkle-seeded, have higher sugar content than at later stages of development.

Green pea flavors are termed "hay like", which can be caused by tissue damage, stimulating enzymatic production of volatiles. Hexanol, which is an end product of lipid degradation, could be of importance in the development of this off flavor (Murray et al., 1976).

Consumer surveys on factors governing vegetable preferences showed that texture and flavor were the most important sensory attributes, followed by appearance and color (Schultz et al., 1984).

The structure of the cell wall of the green pea is a very important contributor to the texture of green peas. In vegetables, tissue differentiation, cell enlargement, and cell wall growth continue

throughout the edible stage. The continued growth and thickening of cell wall eventually involves lignification and secondary cell wall formation, accounting for most of the chemical aspects of the toughening of vegetables (Van Buren, 1979). Therefore, as the peas become overly mature, the seed coats of peas toughen and their quality as a vegetable declines.

High levels of graininess and a mushy consistency of cotyledons and toughness of the seed coat were observed in cooked canned green peas (Grillier, 1989).

Recently, few sensory data have been generated on canned peas. Martens and van der Burg (1984) and Martens (1986) applied multivariate sensory data analysis techniques to characterize frozen peas and for relating instrumental and sensory data from peas. However, no information was available for characterization of the developmental changes associated with common commercial cultivars.

Therefore, the objective of this study is to compare the texture and flavor changes during seed development in four pea cultivars.

MATERIALS AND METHODS

Sample Preparation

Four cultivars of peas, one large wrinkle-seeded (w), two round-seeded (R-1, R-2), and one small-seeded freezer-type (F), were grown on commercial experimental plots in northwestern Illinois. Two plantings were harvested for the study. Peas were monitored until they reached three levels of maturity represented by tenderometer units (TU) of 90, 110, and 120. When fields of peas reached these levels they were harvested with a commercial harvester, taken to a pilot plant canning line, size graded, washed, blanched, packed, brined, sealed and processed to insure commercial sterility in a FMC Steritort. Samples were transported to Ames, IA, and they were stored at $< 25\ ^{o}C$ until analyzed.

Sensory Analyses

The study was conducted in 12 sessions with four samples presented at a session. All samples were presented four times during the study. The textural attributes were firmness, from not firm to very firm; pastiness, from not pasty to very pasty; and tough seedcoats, from very tender to very tough. For the flavor attributes, sweetness was evaluated, from not very sweet to very sweet and green pea flavor, from very little to very much. A 15 cm line scale with anchors, 1.5 cm from each end was used. See Appendix A for more detailed

instructions and definitions for each of these terms. There were thirteen persons on the panel who were trained for each of the attributes, using samples that spanned the range expected in this study. Training samples were manipulated for sweetness. Commercial samples of known textural diversity were used for each of those attributes. Each sample was heated to 80 ^{o}C and served in covered preheated, glass, petri dishes (6.0 x 1.5 cm). Approximately 20 g of peas were served to each panelist. The samples were served sequentially to insure that they remained warm during the evaluation. Panelists were served in individual booths. Cool white fluorescent lights were used in the booths.

Data Analyses

A partially balanced, incomplete block design was carried out. An analysis of variance using the PROC GLM procedure in SAS (1984, 1985) was used to accomplish the statistical analyses.

RESULTS AND DISCUSSION

Instrumental Classification of the Sample Maturity

The original criteria for obtaining maturity classes of peas based on target TU values of 90 (under-), 110 (optimumly-), and 120 or greater (overly-mature) proved too difficult to attain precisely for the early target maturity class due to the imprecision of the Tenderometer system.

Nevertheless, there were three distinct classes of peas sampled for the sensory analyses based on TU values. The early group had mean TU value of 110. The optimum maturity group had a mean TU value of 115. The third group had TU values ranging from 125 to 154 with a mean of 136. These numbers reflect the difficulty of using such an imprecise measuring device to classify the maturity stages in commercial pea samples, and how the TU value for a field run sample will have individual sieve sizes with different TU values as previously pointed out by Voisey and Nonnecke (1973).

Cultivar Differences

General linear model analyses of the sensory scores for textural attributes showed significant cultivar differences for firmness ($p \leq 0.0001$), pastiness ($p \leq 0.0038$), and tough seed coat ($p \leq 0.0001$) (Table 2). Statistical analyses of the individual textural attribute responses showed that the freezer-type cultivar (F) was the most firm,

least pasty, and had the toughest seed coats of the four cultivars (Table 1). These rankings were true for these attributes over all maturity levels. The least firm cultivar was the wrinkle-seeded one (W). The round-seeded cultivars were always the pastiest of the samples analyzed and they had softer seed coats.

The general linear model analyses of the flavor attribute of sweetness showed a significant difference among cultivars ($p \leq 0.0001$) while the green pea flavor attribute was not significantly different at the 5% level ($p \leq 0.0811$) (Table 2). Except for the R-1 cultivar, the perception of sweetness in these samples decreased significantly in all cultivars throughout the study. The wrinkle-seeded sample was judged the sweetest and the freezer-type was scored the least sweet at optimum maturity (Table 4). One of the round seeded samples (R-1) was judged least sweet. For the attribute of green pea flavor, there was an inconsistent pattern in the perception of this flavor attribute. The freezer-type cultivar was scored the highest in green pea flavor at the immature stage (TU $\leq$ 90) and the R-2 cultivar at optimum maturity. No trend in the ranking of the scores was apparent for any variety.

Table 2 summarizes the analyses of variance for the cultivar and maturity effects in this study and significant interactions shown by firmness and seed coat toughness scores.

Maturity Differences

The textural attributes showed some interesting trends as a function of the maturity of the samples. One of the round-seeded cultivars (R-1) softened as the peas matured (Table 3) while the other three cultivars exhibited increased firmness with increasing maturity. Seed coat toughness scores changed in a less consistent fashion due to the statistically significant interaction of cultivar vs. maturity (Table 2). The freezer-type was always evaluated as having the toughest seed coats, and the data indicated that there were no significant differences with increasing maturity. However, the wrinkle-seeded and one of round-seeded cultivars (R-2) were less tough at the optimum maturity (TU = 110) than they had been at maturity 1 (TU = 90). These same cultivars reached their toughest values at the TU value of $\geq$ 120. The other round-seeded cultivar (R-1) exhibited less and less toughness of its seed coats as its maturity increased.

The pastiness attribute significantly increased in all varieties as they matured (Table 2). The freezer-type cultivar, however, did not exhibit a significant increase in this measure until the sample was overly mature (TU $\geq$ 120). The wrinkle-seeded cultivar exhibited a linear increase in the scores for this attribute as the peas matured. The round-seeded cultivar (R-1) was rated initially as the pastiest of all

samples, and the rating continued to increase slightly as the samples matured (Table 3).

The changes in the sweetness flavor attribute with increasing maturity showed significant changes ($p \leq 0.0001$) (Table 2) as well as the cultivar differences shown above. These was no significant trend ($p \leq 0.4321$) related to the changes in green pea flavor as the cultivars matured. Except for the R-1 cultivar, the sweetness scores for all cultivars decreased significantly up to the optimum maturity and remained constant (Table 4). The wrinkle-seeded cultivar showed the highest sweetness scores for all of the maturity levels while the round-seeded cultivar (R-1) had the lowest score initially and changed little as the maturity increased. The freezer-type cultivar had the highest score for green pea flavor; however, the scores at greater maturity did not change much (Table 4). Both of the round-seeded cultivars and the wrinkle-seeded cultivar showed increased green pea flavor with maturity; however, the changes were not significant (Table 2, 4).

Table 1. Sensory data for textural and flavor attributes compared by cultivars

Cultivar[b]	Attributes[a]				
	Firmness	Pastiness	Toughness of seed coats	Sweetness	Green pea flavor
R-1	8.17 ± 0.57[b]	9.52 ± 0.51[b]	6.91 ± 0.42[b]	5.91 ± 0.33[b]	7.07 ± 0.35
R-2	5.84 ± 0.57[c]	8.92 ± 0.51[b]	5.30 ± 0.42[c]	7.05 ± 0.33[c]	7.43 ± 0.35
F	8.14 ± 0.57[b]	6.75 ± 0.51[c]	10.68 ± 0.42[d]	6.16 ± 0.33[b]	7.38 ± 0.35
W	3.59 ± 0.57[d]	7.72 ± 0.51[c]	8.78 ± 0.42[e]	8.76 ± 0.33[d]	6.24 ± 0.35

[a]Mean cultivar score for attribute ± standard error of the mean. Within a column, samples with different letters are significantly different at $p \leq 0.05$. those cultivars without letters are not significantly different ($p \leq 0.05$).

[b]R-1, R-2 = round-seeded cultivars;
F = small freezer-type cultivar;
W = wrinkle-seeded cultivar.

Table 2. Summary of analyses of variance for textural and flavor sensory data in green peas of different maturity

Attribute	Probability		
	Cultivar	Maturity	Cultivar * Maturity Interaction
Firmness	0.0001	NS[a]	0.0335
Pastiness	0.0038	0.0001	NS
Toughness of seed coats	0.0001	0.0315	0.0044
Sweetness	0.0001	0.0001	NS
Green pea flavor	NS	NS	NS

[a]NS = not significantly different (P > 0.05).

Table 3. Comparisons of least square means of the sensory textural attributes (firmness, pastiness, and toughness of seed coats) in four cultivars of green peas at different maturities

Cultivar[a]	Maturity	Firmness	Pastiness	Toughness of Seed Coats
R-1	1	9.99[b]	9.14[c]	9.67[d]
R-1	2	7.46	9.41	5.50
R-1	3	7.08	10.00	5.56
R-2	1	4.03	5.97	5.37
R-2	2	5.27	9.85	3.80
R-2	3	8.22	11.12	6.73
F	1	6.70	5.47	10.26
F	2	8.61	5.74	11.14
F	3	9.12	9.03	10.63
W	1	2.53	5.25	8.97
W	2	3.32	7.94	7.94
W	3	4.91	9.95	9.43

[a]R-1, R-2 = round-seeded cultivars;
F = small freezer-type cultivar;
W = wrinkle-seeded cultivar.
[b]Standard Error of LS Mean = ± 0.99.
[c]Standard Error of LS Mean = ± 0.89.
[d]Standard Error of LS Mean = ± 0.72.

Table 4. Comparison of least square means of the flavor characteristics (sweetness and green pea flavor) in four cultivars of green peas at different maturities

Cultivar[a]	Maturity	Sweetness	Green pea flavor
R-1	1	6.43[b]	6.62[c]
R-1	2	6.02	6.83
R-1	3	5.28	7.76
R-2	1	9.20	6.56
R-2	2	5.79	8.29
R-2	3	6.14	7.44
F	1	7.91	7.65
F	2	4.83	7.20
F	3	5.74	7.30
W	1	10.50	6.02
W	2	8.57	6.26
W	3	7.22	6.42

[a]R-1, R-2 = round-seeded cultivars;
F = small freezer-type cultivar;
W = wrinkle-seeded cultivar.
[b]Standard Error of LS Mean = $\pm$ 0.57.
[c]Standard Error of LS Mean = $\pm$ 0.61.

CONCLUSION

General linear model analyses of the sensory scores for textural attributes showed significant cultivar differences for firmness ($P \leq 0.0001$), pastiness ($P \leq 0.0038$), and tough seed coat ($P \leq 0.0001$).

The freezer type cultivar was the most firm, least pasty, and had the roughest seed coats of the four cultivars over all maturity levels. The rounded seeded cultivars were the pastiest and had softer seed coasts. The pastiness attribute increased in all cultivars as they matured. Each cultivar exhibited different changes in this attribute.

The flavor attribute of sweetness showed a significant difference among cultivars ($P \leq 0.0001$) while the green pea flavor attribute was not significantly different at the 5% level ($P \leq 0.0811$).

The wrinkle-seeded cultivar was judged the sweetest and the freezer-type was scored the least sweet at optimum maturity.

The changes in flavor attributes of sweetness with increasing maturity showed significant changes ($P \leq 0.0001$). The sweetest scores for all cultivars decreased throughout the study. The green pea flavor change showed an inconsistent pattern as the cultivars matured and no significant difference between cultivars or over the three maturities studied.

REFERENCES

Aleksic, G.; and Luh, B. 1975. Grades and standards. In Commercial vegetable processing; Luh, B., Woodorf, J., Eds; AVI Publishing Company, Inc.: Westport, CT, 1975; p. 491-534.

Arthey, D. Vinning peas-processing and marketing. In The pea crop; Hebblethwaite, P., Heath, M., Dawkins, T., Eds; Butterworths: London, 1985; p. 433-440.

Grillier, D. E. Physiochemical and sensory analyses of optimal and extra-standard quality peas. M. S. Thesis, Iowa State University, Ames, IA, 1989 (unpublished).

Martens, M. Sensory and chemical/physical quality criteria of frozen peas studied by multivariate data analysis. J. Food Sci. 186, 3, 599-603.

Martens, M.; van der Burg. Relating sensory and instrumental data from vegetables using different multivariate techniques. In Progress in flavor research; Adda, J., Ed; Elseviev Science Publishers: Amsterdam, 1984; p. 131-148.

Murray, K. E.; Shipton, J.; Whitfield, F. B.; Last, J. H. The volatiles of off-flavored unblanched green peas. J. Sci. Food Agric. 1976, 27, 1093.

SAS-PROCGLM, SAS Institute, Inc., Cary, NC, 1984, 1988.

Schultz, H.; Martens, M.; Rodbottem, M.; Wilshewr, B. Consumer perception of vegetable quality. Acta Hort. 1984, 163, 31-38.

Van Buren, J. P. The chemistry of texture in fruits and vegetables. J. Texture Studies 1979, 10, 1-23.

Voisey, P. W.; Nonnecke, I. L. Measurement of pea tenderness. J. Texture Studies 1973, 4, 323-343.

Part III. THE FLAVOR VOLATILES OF GREEN PEAS (Pisum sativum)

INTRODUCTION

Many biochemical changes occur during the development of the vegetables. Quality was defined by Kramer and Twigg (1966) as the composite of those chemical and physical characteristics that make a product possess consumer appeal and acceptability. One of the most important aspects of these characteristics is flavor.

Commercially produced frozen green peas usually possess a "haylike" off-flavor. Enzymic hydrolysis of lipids and peroxide formation take place in unblanched frozen vegetables and successive reactions may be responsible for the development of off-flavor (Lee and Mattick, 1961; Tappel, 1961). Although a number of constituents of pea volatiles have been identified (Ralls et al., 1965; Whitefield et al., 1966; Murray et al., 1968; Murray et al., 1976), little work has been done to characterize volatiles associated with optimum pea flavor or those volatiles that might be an index to the development of optimal harvest maturity.

Gas chromatography provides a practical means of objective analysis of food aromas. Development of flame ionization detectors enables detection of concentrations as low as parts per billion. The wide choice of temperatures and column coatings, packings, and lengths has enabled separation of compounds with small differences in molecular structure. Rapid, accurate and reproducible analyses can be conducted

under conditions of low temperatures, inert atmospheres and small sample size. Consequently, food scientists attemot to correlate gas chromatograms of foods (Pesek, 1984).

Chemical and gas chromatographic (GC) studies have reported volatile components associated with processing and storage of peas. Bengtsson and Bosund (1964) used gas chromatography to evaluate the formation of volatile substances and Ralls et al. (1965) combined gas chromatography and mass spectrometry to identify 40 components in peas. However, combined gas chromatography - mass spectrometry (GC-MS) can be impaired by 1) incomplete resolution of components, 2) distortion of the spectrum due to the inevitable rapid change in concentration, and 3) interference from the background spectrum caused by phase bleed from the column (Murray et al., 1968).

The present investigation was conducted to identify the volatile constituents which change significantly during development and might be used as an index to maturity. These changes could also serve as an index to physiological events important to agricultural scientists involved in pea quality enhancement.

MATERIALS AND METHODS

Materials

Seven commercial cultivars of green peas (Pisum sativum) were grown in the greenhouse under proper conditions. Light, ventilation, and temperature were adjusted to obtain optimal growth. Soils were checked weekly to maintain desirable pH (6.0-6.5) and nutrient content. The day of fertilization was tagged with a Julian date when each flower's first petal was reflexed, and the plants were grown to near senescence. Samples were harvested, separated by day after fertilization (DAF) from 12 to 22, separated by sieve size, frozen with liquid nitrogen and stored in the freezer at -75 ^{o}C. Where material was available, duplicate analyses were made for samples in these maturity periods, early (12-14 DAF), middle (15-17 DAF), and late (18-22 DAF).

Volatile Analyses

The peas were thawed and homogenized with deionized water (9:1 ratio) with a Tekmar tissuemizer for 1 min. Duplicate 5 g portions of each sample were transferred to separate reaction vials. Vials were sealed using Teflon coated septa and aluminum seals (Supelco). All vials were allowed to equilibrate at 37 ^{o}C in an oven for 30 min.

Before sample injection, the first loop of the column was submerged into liquid nitrogen and sample vials were removed from

the oven, placed in a styrofoam insulated container, and carried to the GC. A gas tight syringe was flushed with sample headspace three times before 2 ml of headspace were injected (Wilson et al., 1989).

The loop of the GC column was removed from the nitrogen after 1 minute to begin the temperature programmed analyses.

GC Analyses

Volatile analyses were performed on a Varian 3470 gas chromatograph (GC) equipped with a Hewlett Packard 3390A reporting integrator. The column was a 30 meter fused-silica DB-5 with an internal diameter of 0.25 mm and a 0.1 μ film thickness. The injector and detector were maintained at 150 ^{o}C and 250 ^{o}C, respectively.

The column oven temperature was held at 40 ^{o}C after injection of a sample, increased 10 ^{o}C per min for 20 min to 230 ^{o}C, reset to 40 ^{o}C, and left to equilibrate before injection of the next sample. Carrier gas flow was 1.49 mL/min with a split ratio of 10:1 and splitter was turned on 1 min after injection.

Compound Identification

Major compounds were identified by (1) retention times compared with standards, (2) Kovats indices (Jennings and Shibamoto, 1980), and (3) GC-Mass spectroscopy.

GC-MS analyses were made by the Chemistry Instrument Services, Iowa State University. A Finnigan 4000 GC-MS with DB-5 column

and the Incos data system provided tentative compound identification. The column oven temperature was held at 50 °C for 1 minute and programmed to 230 °C. Mass spectral database and "National Bureau of Standard Subset Library" (Heller and Milne, 1978) were used.

Statistical Analyses

The Statistix analytical software package (version 3.0) was used in all statistical analyses (Statistix, 1989).

RESULTS AND DISCUSSION

The volatile compounds were identified by comparison of mass spectra, chemical standards, and GC retention times. Compounds which agreed with a minimum of two comparisons were considered to be identified. Compounds identified with a single reference comparison were designed to be tentatively identified. Nine compounds were identified and twelve tentatively identified (Table 1). Except for the alkane, nonane, all compounds have been reported previously (Ralls et al., 1965; Whitfield and Shipton, 1966; Murray et al., 1976; Murray et al., 1968).

Selected major volatiles from seven green pea cultivars at different maturity stages are shown in Table 2. For all cultivars, the most abundant compounds were hexanal, n-nonane, methyl heptanoate, n-undecane, and propyl benzoate. Both of the round-seeded cultivars had losses of these major volatile compounds as they matured.

The freezer-type cultivar and one of wrinkle-seeded cultivars (W-1) exhibited a similar pattern of volatile change. For these cultivars, all of the major volatiles except hexanal increased with increasing maturity. For one of the wrinkle-seeded cultivars (W-2), the content of hexanal and n-nonane increased while n-undecane and propyl benzoate decreased. However, in cultivar W-4, the opposite changes were seen. Methyl heptanote remained constant in these two cultivars (Table 2).

It has been reported that the volatile constituents of peas include a complex mixture of aliphatic and aromatic hydrocarbons (Ralls et al., 1965; Murray et al., 1976). However, the origins of aliphatic and aromatic hydrocarbons in peas are not well known. Murray et al. (1976) assumed three possible causes: (a) adsorption of hydrocarbon plant constituents from the atmosphere or from decaying plant remains, (b) absorption of petroleum hydrocarbons used in pesticide sprays or fuels employed in agricultural production, (c) the breakdown in the soil environment of plant carotenoids to aromatic hydrocarbons. The origins of n-nonane and n-undecane, major volatiles found in present study, could likely come from such sources.

Methyl heptanoate and propyl benzoate were reported as two major volatiles in this study. Phenylalanine was a likely precursor of the oxygenated aromatic compounds of the pea, namely, benzaldehyde, acetophenone, ethyl phenylacetaldehyde, acetophenone, ethyl phenylacetate, ethyl cinnamate and the benzoate esters, according to Murray et al. (1976). Eriksson (1973) reported that the aliphatic esters might be considered secondary products of lipid oxidation.

The origin of the carbonyl volatiles could be derived from either enzymatic or autoxidative decomposition of fatty materials in peas. Unblanched peas deteriorate in flavor when held in the frozen stage due to naturally occurring enzymes not inhibited by low temperature

(Lee and Wagenknecht, 1951). They later reported that adding enzymes to blanched peas induced the development of a flavor change similar to off-flavor (Lee and Wagenknecht, 1958). Whitfield and Shipton (1966) concluded enzymatic oxidation of the unsaturated fatty acids could be favored by a low storage temperature because low temperature autoxidation of fat was negligible in comparisons with enzymatic oxidation (Siddiqi and Tappel, 1956). Volatiles in frozen unblanched peas stored 8 months at various temperatures were studied by Bengtsson and Bosund (1964). The amount of hexanal increased rapidly with storage temperature.

It has been reported that lipoxidase was present in peas (Siddiqi and Tappel, 1956; Lee and Wagenknecht, 1958). It was shown that enzymatic hydrolysis of lipids and peroxide formation took place in unblanched frozen vegetables and that successive reactions may be responsible for the development of off-flavor (Lee and Mattick, 1961).

Hexanal, a major volatile compound in the present work, was reported in unblanched frozen peas by Bengtsson and Bosund (1964). They suggested that reactions following the oxidation of linoleic acid by lipoxidase may account for the formation of hexanal.

Summations of peak areas for the selected total volatiles from seven cultivars of green peas were shown in Table 3. Retention times for volatile compounds contributing to these summations ranged from

2.8 min to 13.3 min. Each cultivar showed a decrease in the total volatile contents while two of the wrinkle-seeded cultivars (W-2, W-4) changed inconsistently. However, the changes occurred inconsistently in the area of each peak and the numbers of total peaks for any given cultivar.

Table 1. Volatile compounds identified from cryogenic focusing analyses of seven cultivars of green peas

Compound[a]	Indentification		
	CS[b]	KI[c]	MS[d]
n-pentanol		+	+
4-methyl-2-pentanol		+	
hexanal		+	+
cis-3-hexanol		+	+
n-hexanol		+	+
n-heptan-2-o1		+	
n-nonane	+	+	
benzaldehyde	+	+	
n-heptanol		+	
octan-2-o1		+	
methyl heptanoate		+	
1,8-cineole	+	+	
trans-2-octenal		+	
acetophenone		+	
n-octanol		+	
n-undecane	+	+	
ethyl benzoate		+	
n-dodecane	+	+	
propyl benzoate		+	
n-butyl benzoate		+	
diphenyl		+	

[a]Compounds listed in increasing order of retention time.
[b]Chemical standard.
[c]Kovat Index.
[d]Mass spectrometry.

Table 2. Selected major volatile changes from seven cultivars of green peas at different maturity stages

Cultivar[a]	DAF[b]	Peak area of major compounds[c]				
		hexanal	n-nonane	methyl heptanoate	n-undecane	propyl benzoate
R-1	16	2.4	2.3	3.3	1.0	3.5
	20	0.2	1.3	2.1	0.6	4.4
R-2	13	1.1	3.1	2.6	4.4	3.9
	18	0.4	1.6	1.6	3.3	3.4
F	16	2.4	1.3	2.9	5.3	5.2
	20	0.7	2.4	3.1	5.8	6.5
W-1	16	2.7	2.9	3.4	6.7	5.1
	20	1.5	3.3	4.3	6.8	5.5
W-2	12	2.0	2.8	4.4	10.2	9.0
	16	9.3	6.7	4.3	7.8	6.4
W-3	15	1.6	6.7	9.1	14.0	11.3
	19	2.1	2.8	7.2	12.5	11.1
W-4	15	1.9	5.6	10.7	11.7	10.4
	20	1.1	4.2	9.0	13.3	11.9

[a]R-1, R-2 = round-seeded cultivars;
F = small freezer-type cultivar;
W-1, W-2, W-3, W-4 = wrinkle-seeded cultivars.
[b]DAF = days after fertilization.
[c]Peak area x 10^4 (n=2).

Table 3. Changes in selected total volatile contents of green peas at three different days after fertilization (DAF)

Cultivar[a]	Maturity[b,c]		
	Early	Middle	Late
R-1	--	7.6	3.9
R-2	10.5	--	3.2
F	--	11.3	4.4
W-1	--	8.2	8.0
W-2	10.4	15.3	10.6
W-3	12.6	12.6	8.8
W-4	3.0	8.3	7.8

[a]R-1, R-2 = round-seeded cultivars;
F = small freezer-type cultivar;
W-1, W-2, W-3, W-4 = wrinkle-seeded cultivars.
[b]Maturity(Early = 12-14 DAF, Middle = 15-17 DAF, Late = 18-22 DAF).
[c]Mean peak area x 10^5(n = 2).

CONCLUSION

For all cultivars, the most abundant volatile compounds were hexanal, n-nonane, methyl heptanoate, n-undecane, and propyl benzoate.

Both of the round-seeded cultivars had losses of these major volatile compounds as they matured. All of major volatiles except hexanal increased with increasing maturity in the freezer-type cultivar and one of wrinkle-seeded cultivars (W-1). For the W-2 cultivar, the content of hexanal and n-nonane increased while n-undecane and propyl benzoate decreased. However, in cultivar W-4, the opposite changes were seen.

Each cultivar showed a decrease in the selected volatile contents while two wrinkle-seeded cultivars (W-2, W-4) changed inconsistently.

There was no single volatile which could be used to indicate harvestable maturity.

Four of these seven cultivars, R-1, R-2, F and W, were grown commerically, thermally procesed, and subjected to analyses by a trained sensory panel utilizing quantitative descriptive analyses. Samples in Part II had maturities equivalent to the oldest samples from the green house study and some of these DAF's were > 21-22. During these time frames, the green pea flavor results showed no appreciable change. From the GC volatile analyses, the green pea flavor/odor compounds were decreasing for 3 of these cultivars. The wrinkle-seeded cultivar

showed a 4 fold increase at 20 days after fertilization; however, the sensory panel could not perceive any differences in the processed forms of this cultivar. Its green pea floavor scores given by the panel were not different from the other three cultivars.

REFERENCES

Bengtsson, B.; Bosund, I. Gas chromatographic evaluation of volatile substances in stored. peas. Food Technol. 1964, 18, 773.

Heller, S. R.; Milne, G. W. A., Eds. EPA/NIH mass spectral data base; National Bureau of Standards: Washington, D.C., 1978.

Kramer, A.; Twigg, B. A., Eds. Fundamentals of quality control for the food industry; Avi Publishing: Westport, CT, 1966.

Lee, F. A.; Mattick, L. R. Fatty acids of the lipids of vegetables. I. Peas (Pisum sativum). J. Food Sci. 1961, 26, 273.

Lee, F. A.; Wagenknecht, A. C. On the development of off-flavour during the storage of frozen raw peas. Food Research 1951, 16, 239.

Lee, F. A.; Wagenknecht, A. C. Enzyme action and off-flavour in frozen peas. II. The use of enzymes prepared from garden peas. Food Research 1958, 23, 584.

Murray, K. E.; Shipton, J.; Whitfield, F. B.; Kennett, B. H.; Stanley, G. Volatile flavor components from green peas (Pisum sativum). 1. Alcohols in unblanched frozen peas. J. Food Sci. 1968, 33, 290.

Murray, K. E.; Shipton, J.; Whitfield, F. B.; Last, J. H. The volatiles of off-flavored unblanched green peas (Pisum sativum). J. Sci. Food Agric. 1976, 27, 1093.

Pesek, C. A. Spice quality: A comparison of grinding techniques and methods of determining quality. M. S. Thesis, Parks Library, Iowa State University, Ames, IA, 1984.

Ralls, J. W.; McFadden, W. H.; Siefert, R. M.; Black, D. R.; Kilpatrick, P. W. Volatiles from a commercial pea blancher. Mass spectral identification. J. Food Sci. 1965, 30, 228.

Siddiqi, A. M.; Tappel, A. L. Catalysis of linoleate oxidation by pea lipoxidase. Arch. Biochem. Biophys. 1956, 60, 91.

Tappel, A. L. Biocatalysis: lipoxidase and hematin compounds. In Autoxidation and antioxidants; Lundberg, W. O., Ed.; Interscience, New York, 1961; p. 325.

Whitfield, F. B.; Shipton, J. Volatile carbonyls in stored unblanched peas. J. Food Sci. 1966, 31, 328.

Wilson, L. A.; Turner, W. D.; Wilson, W. D.; Pesek, C. A.; Ducan, H. J. Analytical methods for the evaluation of spice quality; 198th National American Chemical Society Meeting: Miami Beach, FL, 1989.

GENERAL SUMMARY AND CONCLUSIONS

Pectinmethylesterase (PME) decreased over all cultivars and all sieve sizes. The mean rate of activity loss followed an apparent first-order rate model. The 'k' (apparent first order rate constant) was 0.168 $(Day)^{-1}$. The freezer-type cultivar (F) had the highest k ($k=0.25$); whereas, one of the round-seeded cultivars (R-2) had the lowest rate ($k=0.11$). A three fold change in PME specific activity was seen over the maturity levels for an individual cultivar and across all cultivars.

All cultivars exhibited the accumulation of the salt-soluble protein 2-4 times as their maturity increased. As the peas seeds matured, they accumulated these proteins. Salt soluble proteins are indicative of globulins and not albumins, which are largely enzymes.

Each cultivar contained different amounts of pectin. Comparing changes in chelate soluble pectin over the time of the study, two of the wrinkle-seeded cultivars showed changes of less than 20% (W-2, W-3). The greatest decreases of chelate soluble pectin were exhibited by R-2 and W-1.

The moisture level was inversely related to the maturity level. Each seed type showed either a biphasic or a linear relation between dry matter accumulation and the maturity index (DAF).

General linear model analyses of the sensory scores for textural attributes showed significant cultivar differences for firmness ($P \leq 0.0001$), pastiness ($P \leq 0.0038$), and tough seed coat ($P \leq 0.0001$). The freezer-type was the most firm, least pasty, and had the toughest seed coats. The round seeded cultivars were always the pastiest and had softer seed coats.

The flavor attribute of sweetness showed a significant difference among cultivars ($P \leq 0.0001$) as well as maturities ($P \leq 0.0001$). The wrinkle-seeded cultivar was judged the sweetest, and the freezer-type was scored the least sweet at optimum maturity. Generally, the sweetness scores for all cultivars decreased as maturity increased. There was an inconsistent pattern in the perception of green pea flavor among cultivars and maturities.

For all cultivars, the most abundant volatile compounds were hexanal, n-nonane, methyl heptanoate, n-undecane, and propyl benzoate. However, each cultivar showed a different pattern of change during the development. There was no single volatile that could be used to indicate harvestable maturity.

RECOMMENDATIONS

The activity of Pectin methyl esterase was measured by the continuous spectrophotometric assay. Attempts should be made to compare the PME activity by several methods. The amount of cellulose and hemicellulose should be determined, which are important contributors to the texture of green peas. Research on the changes in starch accumulation and sugar loss is required because the correct time for harvesting is important. Calcium changes in these cultivars should be analyzed to determine any potential for crosslinkage of the pectin by calcium.

GENERAL REFERENCES

Adlard, E. R.; Bowen, A. W.; Salmon, D. G. Automatic system for the high resolution gas-chromatographic analysis of gasoline range hydrocarbon mixtures. J. Chromatogr. Sci. 1979, 186, 207.

Alberscheim, P. Instability of pectin in neutral solutions. Biochem. Biophys. Res. Commun. 1959, 1, 253-256.

Albersheim, P. Concerning the structure and biosynthesis of the primary cell walls of plants. Int. Rev. Biochem. 1978, 16, 127-150.

Arthey, D. Vinnig peas-processing and marketing. In The pea crop; Hebblethwaite, P., Heath, M., Dawkins, T., Eds; Butterworths: London, 1985; p. 433-440.

Bartley, I. M.; Knee, M. The chemistry of textural changes in fruit during storage. Food Chem. 1982, 9, 47-58.

Beevers, L.; Poulson, R. Protein synthesis in cotyledons of Pisum sativum L. I. changes in cell free amino acid incorporation capacity during seed development and maturation. Plant Physiol. 1972, 49, 476-481.

Blumenkrantz, N.; Asboe-Hansen, O. G. New method for quantitative determination of uronic acids. Anal. Biochem. 1973, 54, 484-489.

Bourne, M. C. Texture of fruits and vegetables. In Rheology and texture in food quality; deMan, J. M., Voisey, P. W., Rasper, V. F., Stanley, D. W., Eds; AVI Publishing Company, Inc.: Westport, CT, 1976; p. 275-307.

Brandt, L. M.; Jeltema, M. A.; Zabik, M. E.; Jeltema, B. D. Effects of cooking in solutions of varying pH on the dietary fiber components of vegetables. J. Food Sci. 1984, 49, 900.

Brettel, T. A.; Grob, R. L. Cryogenic techniques in gas chromatography (Part One). American Laboratory 1985, 17, 19.

Brettel, T. A.; Grob, R. L. Cryogenic techniques in gas chromatography (Part Two). American Laboratory 1985, 17, 50.

Bryant, J. Differentiation of storage cells in seeds of legumes. In Quality in stored and processed vegetables and fruits; Goodenough, P., Atkin, R., Eds; Academic Press: London, 1981; p. 193-201.

Burrows, W. J.; Carr, D. J. Cytokinin content of pea seeds during their growth and development. Physiologia Pl. 1970, 23, 1064-1070.

Candy, D. J., Ed. Biological functions of carbohydrates; John Wiley and Sons: New York, 1980; p. 130-151.

Carr, D. J.; Skene, K. G. M. Diauxic growth curve of seeds, with special reference to French beans. Aust. J. Biol. Sci. 1961, 14, 12.

Cert, A.; Bahima, J. Headspace sampling and G C analysis of volatile urinary metabolites. J. Chromatogr. Sci. 1984, 22(1), 7.

Charlambous, G., Ed. Analysis of foods and beverages. Academic Press: New York, 1978.

Cox, R. D.; Earp, R. F. Determination of trace level organics in ambient air by high resolution gas chromatography with simultaneous photoionization and flame ionization detection. Anal. Chem. 1982, 54 (13), 2265.

Dainty, J.; Hope, A. B.; Denby, C. Ionic relations of cells in Chara australis. Austral. J. Biol. Sci. 1960, 13, 267.

Dische, Z. A new specific color reaction of hexuronic acids. J. Biol. Chem. 1947, 167, 189-198.

Emberger, R. An analytical approach to flavor research. Cereal Foods World 30, 1985, 10, 691-694.

Flinn, A. M.; Pate, J. S. Biochemical and physiological changes during maturation of fruit of the field pea (Pisum arvense L.). Ann. Bot. 1968, 32, 479-495.

Fox, D. J.; Thurman, D. A.; Boulter, D. Studies on the proteins of seeds of the Leguminosae. I. Albumins. Phytochemistry 1964, 3, 417-419.

Frank, H. S. The structure of water. Federation Proc. 1965, 24, 5.1-5.11.

Goodwin, T. W.; Mercer, E. I., Eds. In Introduction to plant biochemistry, 2nd ed; Pergamon Press: New York, 1983; p. 55-91.

Gould, S. E. B.; Rees, D. A.; Richardson, N. G.; Steele, I. W. Pectic polysaccharides in the growth of plant cells: Molecular structural factors and their role in the germination of white mustard. Nature 1965, 208, 876-878.

Grant, G. T.; Morris, E. R.; Rees, D. A.; Smith, P. J. C.; Thom, D. Biological interactions between polysaccharides and divalent cations: The egg-box model. FEBS Letters 1973, 32, 195-198.

Hall, M. A. 1981. Cell wall structure in relation to texture. In Quality in stored and processed vegetables and fruit; Goodenough, P. W., Atkin, R. K., Eds; Academic Press: London, 1985; p. 53-64.

Hartmann, H. T.; Kofranek, A. M.; Rubatzky, V. E.; Flocker, W. J. Plant science. Prentice Hall, NJ, 1988; p. 541.

Heath, H. B.; Reineccius, G., Eds. Flavor chemistry and technology; AVI Publishing Company: Westport, CT, 1986.

Hedley, C. L.; Smith, C. M. Genetic variation for pea-seed development. In The pea crop; Hebblethwaite, P. D., Heath, M. C., Dawkins, T. C. K., Eds; Butterworths: England, 1985; p. 329.

Joslyn, M. A. The chemistry of protopectin: A critical review of historical data and recent developments. Adv. Food Res. 1962, 11, 1-107.

Judge, D. P., Ed. The almanac of canning, freezing, preserving industries; Edward E. Judge & Sons, Inc.: Westminster, 1988.

Keegstra, K.; Talmadge, K. W.; Bauer, W. D.; Albersheim, P. The structure of plant cell walls. III. A model of the walls of suspension-cultured sycamore cells based on the interconnections of the macromolecular components. Plant Physiol. 1973, 51, 188-196.

Keijbets, M. J. H.; Pilnik, W. β-Elimination of pectin in the presence of anions and cations. Carbohydr. Res. 1974, 33, 359-362.

Khvostova, V. V., Ed. Genetics and breeding of peas; Amerind Publishing Co.: New Delhi, 1983.

Kintner, P. K.; Van Buren, J. P. Carbohydrate interference and its correction in pectin analysis using the m-hydroxydiphenyl method. J. Food Sci. 1982, 47, 756-759, 764.

Krost, K. J.; Pellizzari, E. D.; Walburn, S. G.; Hubbard, S. A. Collection and analysis of hazardous organic emissions. Anal. Chem. 1982, 54(13), 2265.

Lamport, D. T. A. Cell wall metabolism. Ann. Rev. Plant Physiol. 1970, 21, 235-270.

Lamport, D. T. A.; Katona, L; Roerig, S. Galactosylserine in extension. Biochem J. 1973, 133, 125-131.

Lamport, D. T. A.; Miller, D. H. Hydroxyproline arabinosides in the plant kingdom. Pl. physiology 1971, 48, 454-456.

Landry, J.; Moureaux, T. Heterogeneite des glutelines du grain de mais. Bull. Soc. Chim. Biol. 1970, 52, 1021.

Lee, C. Y.; Bourne, M. C.; Van Buren, J. P. Effect of blanching temperatures on the firmness of carrots. J. Food Sci. 1979, 44, 615.

Loh, J.; Breene, W. M. Between species differences in fracturability loss: Comparison of the thermal behavior of pectic and cell wall substances in potato and Chinese waterchestnut. J. Texture Stud. 1982, 13, 381-396.

Makasheva, R. K., Ed. The pea; Amerind Publishing Company; New Delhi, 1983.

McFeeters, R. F.; Armstrong, S. A. Measurement of pectin methylation in plant cell walls. Anal. Biochem. 1984, 139, 212-217.

McNeil, M.; Darvill, A. G.; Albersheim, P. The structural polymers of the primary cell walls of dicots. Prog. Chem. Org. Nat. Prod., 1979, 37, 191-249.

Miller, L.; Macmillan, J. D. Purification and pattern of action of pectinesterase from fusarium oxysporum f. sp. vasinfectum. Biochemistry. 1971, 10, 570-576.

Muhlethaler, K. Ultrastructure and formation of plant cell walls. Ann. Rev. Plant Physiol. 1967, 18, 1-24.

Nonnecke, I, Ed. Vegetable production; AVI Publishing Co.: New York, NY. 1989; p. 251-293.

Northcote, D. H. The cell walls of higher plants: Their composition, structure and growth. Biol. Rev. 1958, 33, 53-102.

Northcote, D. H. Chemistry of the plant cell wall. Ann. Rev. Plant Physiol. 1972, 23, 113-132.

Osborne, T. B., Ed. The vegetable proteins, 2nd Ed., Longmans Green and Co.: London, 1926.

Osjord, E. H.; Malthe-Soerenssen, D. Quantitative analysis of natural gas in a single run by the use of packed and capillary columns. J. Chromatogr. 1983, 279, 219.

Personius, C. J.; Sharp, P. F. Adhesion of potato-tissue cells as influenced by pectic solvents and precipitants. Food Res. 1939, 4, 299-307.

Pomeranz, Y.; Meloan, C., Eds. Food analysis theory and practice; AVI Publishing Co. Inc.: Westport, CT. 1987.

Preston, R. D., Ed. The physical biology of plant cell walls; Chapman and Hall: London, 1974.

Pruthi, J. S., Ed. Spices and condiments: chemistry, microbiology, technology; Academic Press: NY, 1980.

Rees, D. A. Polysaccharide gels A molecular view. Chem. Ind. 1972, 16, 630-635.

Rees, D. A., Ed. Polysaccharide shapes; Halsted Press: New York, NY, 1977.

Rees, D. A.; Welsh, E. J. Secondary and tertiary structure of polysaccharides in solutions and gels. Angew. Chem. Int. Ed. Engl. 1977, 16, 214-224.

Rees, D. A.; Wight, J. N. Molecular cohesion in plant cell walls. Biochem. J. 1969, 115, 431-439.

Ring, S. G.; Selvendran, R. R. An arabinogalactoxyloglucan from the cell wall of Solanum tuberosum. Phytochemistry 1981, 20, 2511-2519.

Rockland, L. B.; Jones, F. T. Scanning electron microscope studies on dry beans. Effects of cooking on the cellular structure of cotyledons in rehydrated large lima beans. J. Food Sci. 1974, 39, 342-346.

Saleeb, F. Z.; Pickup, J. G. Flavor interactions with food ingredients from headspace analysis measurements. In Flavor of foods and beverages; Charalambous, G., Inglett, G. E., Eds.; Academic Press: New York, 1978.

Schultz, H.; Martens, M.; Rodbottom, M.; Wilshewr, B. Consumer perception of vegetable quality. Acta Hort. 1984, 163, 31-38.

Smidsrod, O.; Haug, A. Dependence upon the gel-sol state of the ion-exchange properties of alginates. Acta Chem. Scand. 1972, 26, 2063-2074.

Sterling, C. Effect of solutes and pH on the structure and firmness of cooked carrot. J. Food Technol. 1968, 3, 367-371.

Sterling, C. Effect of moisture and high temperature on cell walls in plant tissues. Food Res. 1955, 20, 474-479.

Takeoka, G; Jennings, W. Developments in the analysis of headspace volatiles. J. Chromatogr. Sci. 1984, 22, 177.

Talmadge, K. W.; Keegstra, K.; Bauer, W. D.; Albersheim, P. The structure of plant cell walls. I. The macromolecular components of the walls of suspension-cultured sycamore cells with a detailed analysis of the pectic polysaccharides. Plant Physiol. 1973, 51, 158-173.

Teranishi, R.; Issenberg, P.; Hornstein, I.; Wick, E. L., Eds. Flavor research principles and techniques; Marcel Dekker, Inc.: New York, 1971.

Thompson, B., Ed. Fundamentals of gas analysis by gas chromatography; Varian Associates, Inc.: Palo Alto, CA, 1977.

Valenzuela, L. H. Edible pea pod production in California. Univ. of Calf. Div. ANR Leaflet 21328, 1983.

Van Buren, J. P. The chemistry of texture in fruits and vegetables. J. Texture Stud. 1979, 10, 1-23.

Van Buren, J. P. Softening of cooked snap beans and other vegetables in relation to pectins and salts. In ACS Symp. Ser. Am. Chem. Soc. Washington, D.C.: The Society 310, 1986; p. 190-199.

Van Buren, J. P.; Moyer, J. C.; Robinson, W. B. Pectin methylesterase in snap beans. J. Food Sci. 1962, 27, 291-294.

Voisey, P.; Nonnecke, I. Measurement of pea tenderness. J. Texture Studies. 1973, 4, 171-195.

Walton, P. D. The grain products. In Principles and practices of plant science; Walton Eds; Prentice Hall: NJ, 1988; p. 291.

Whitaker, J. R., Ed. Principles of enzymology for the food sciences; Marcel Dekker, Inc.: New York, 1972; p. 469-479.

Wright, D. J. Combining peas for human consumption. In Pea crop; Hebblethwaite, P. D., Heath, M. C., Dawkins, T. C. K., Eds; Butterworths: England, 1985; p. 441.

Wood, P. J.; Siddiqui, I. R. Determination of methanol and its application to measurement of pectin ester content and pectin methyl esterase activity. Anal. Biochem. 1971, 39, 418-428.

Zapsalis, C.; Beck, R., Eds. Food chemistry and nutritional biochemistry; John Wiley & Sons, Inc.: New York, 1985; p. 315-414.

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APPENDIX A: Score card for sensory analyses by the trained panel

Panelist No._____

Date_____

Sample No._____

Score Card for Canned Green Peas

Samples of canned peas are to be evaluated for several texture and flavor attributes. Make a vertical mark on the line scale at the point that corresponds to the intensity of the characteristic. Write the code number of the sample by the mark.

Evaluate a spoonful of sample for the following texture characteristics:

FIRMNESS--The force required to bite through the sample using the molar teeth (a sample that requires much force is very firm; soft samples do not resist).

very soft — very firm

PASTINESS--A denseness that persists as you chew the sample.

not pasty — very pasty

TOUGHNESS OF SEEDCOATS--Tough seedcoats resist breakdown as you chew.

very tender — very tough

COMMENTS:

Chew the samples and evaluate for the following flavor characteristics:

SWEETNESS--Sweet flavor characteristic of some canned vegetables.

not sweet — very sweet

GREEN PEA FLAVOR--A 'green' flavor note.

very little — very much

COMMENTS:

APPENDIX B: Statistical analyses of texture and flavor characteristics

Table B.1. Statistical analysis of firmness in four cultivars of green peas

Dependent Variable: FRM

Source	DF	Sum of Squares	Mean Square	F Value	PR > F	R-Square	C.V.
Model	22	214.67	9.75	3.01	0.0044	0.7262	27.9499
Error	25	80.91	3.23		Root Mse		FRM Mean
Corrected Total	47	295.59			1.79		6.44

Source	DF	Type I SS	F Value	PT > F	DF	Type III SS	F Value	P R > F
Block	11	12.80	0.36	0.9603	11	54.87	1.54	0.1786
VAR	3	130.59	13.45	0.0001	3	130.81	13.47	0.0001
MAT	2	17.59	2.72	0.0855	2	17.59	2.72	0.0855
VAR MAT	6	53.68	2.76	0.0335	6	53.68	2.76	0.0335

Table B-2. Statistical analysis of pastiness in four cultivars of green peas

Dependent Variable: PASTI

Source	DF	Sum of Squares	Mean Square	F Value	PR > F	R-Square	C.V.
Model	22	216.10	9.82	3.77	0.0009	0.768210	19.6370
Error	25	65.20	2.60		Root Mse		PASTI Mean
Corrected Total	47	281.30			1.61		8.2241

Source	DF	Type I SS	F Value	PT > F	DF	Type III SS	F Value	PR > F
Block	11	50.13	1.75	0.1200	11	30.69	1.07	0.4216
VAR	3	45.28	5.79	0.0038	3	43.37	5.54	0.0047
MAT	2	91.28	17.50	0.0001	2	91.28	17.50	0.0001
VAR MAT	6	29.39	1.88	0.1244	6	29.39	1.88	0.1244

Table B-3. Statistical analysis of the toughness of the seedcoats in four cultivars of green peas

Dependent Variable: TSC

Source	DF	Sum of Squares	Mean Square	F Value	PR > F	R-Square	C.V.
Model	22	298.51	13.56	7.89	0.0001	0.874090	16.5692
Error	25	43.00	1.72		Root Mse		TSC Mean
Corrected Total	47	341.51			1.311		7.9152

Source	DF	Type I SS	F Value	PT > F	DF	Type III SS	F Value	PR > F
Block	11	83.66	4.42	0.0010	11	23.41	1.24	0.3146
VAR	3	157.36	30.50	0.0001	3	151.98	29.45	0.0001
MAT	2	13.69	3.98	0.0315	2	13.69	3.98	0.0315
VAR MAT	6	43.78	4.24	0.0044	6	43.78	4.24	0.0044

Table B-4. Statistical analysis of sweetness in four cultivars of green peas

Dependent Variable: SWT

Source	DF	Sum of Squares	Mean Square	F Value	PR > F	R-Square	C.V.
Model	22	122.27	5.55	5.13	0.0001	0.818592	14.9354
Error	25	27.09	1.08		Root Mse		SWT Mean
Corrected Total	47	149.37			1.04		6.9707

Source	DF	Type I SS	F Value	PT > F	DF	Type III SS	F Value	PR > F
Block	11	13.97	1.17	0.3534	11	54.88	4.60	0.0008
VAR	3	46.49	14.30	0.0001	3	45.65	14.04	0.0001
MAT	2	48.78	22.50	0.0001	2	48.78	22.50	0.0001
VAR MAT	6	13.01	2.00	0.1034	6	13.01	2.00	0.1034

Table B-5. Statistical analysis of green pea flavor in four cultivars of green peas

Dependent Variable: GRN

Source	DF	Sum of Squares	Mean Square	F Value	PR > F	R-Square	C.V.
Model	22	26.95	1.22	1.01	0.4862	0.470761	15.6609
Error	25	30.30	1.21		Root Mse		GRN Mean
Corrected Total	47	57.26			1.1009		7.0301

Source	DF	Type I SS	F Value	PT > F	DF	Type III SS	F Value	PR > F
Block	11	9.82	0.74	0.6948	11	10.26	0.77	0.6655
VAR	3	9.15	2.52	0.0811	3	8.60	2.37	0.0951
MAT	2	2.10	0.87	0.4321	2	2.10	0.87	0.4321
VAR MAT	6	5.87	0.81	0.5738	6	5.87	0.81	0.5738